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INFLUENCE OF LIGHT, TEMPERATURE, AND SOIL MOISTURE ON THE HARDENING PROCESS IN ALFALFA[1]

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INTRODUCTION

Although much progress has been made in increasing the ability of plants to withstand cold by selection and breeding, little is known of the fundamental physiological phenomena which serve to make a plant "winter hardy." In fact, these improvements have been made by plant breeders under the handicap of having to wait for "test" winters to eliminate the unhardy selections. In any improvement program involving winter hardiness a simple method of determining cold resistance would be of great value, for with such a method newly introduced varieties could be tested without delay.

Studies of cold resistance with the aid of controlled freezing methods have gone forward in various laboratories with promising results. Some workers in this field, notably Hill and Salmon (*7*),[3] Akerman (*1*), and Martin (*11*), have suggested that it is essential for plants to go through the hardening process before correct varietal differentiation in cold resistance can be obtained. The influence of such factors as light, moisture, and even temperature on the ability of plants to resist cold is, however, still largely unknown. Very little is known regarding the time necessary for optimum hardening under uniform conditions. A better understanding of these and other factors would lead to greater refinements in the procedure of determining hardiness and might well be made one of the first steps in a study of the whole problem of winter hardiness.

The present paper deals with the influence of light, temperature, and soil moisture on the ability of alfalfas to survive cold. In practically all the tests several varieties[4] having a wide range in winter

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[3] Reference is made by number (italic) to Literature Cited, p. 514.

[4] It is recognized that the term "variety" is not used in the correct botanical sense. However, since the alfalfas employed in this study have distinct physiological differences and since the term is convenient, they will be termed "varieties" throughout this paper.

hardiness were used with the object of determining what particular set of environmental conditions would bring out the greatest varietal differentiation.

The effect of low temperature on plants has been studied for at least 200 years, and voluminous literature on the subject has accumulated. The work of previous investigators has been reviewed by Blackman (*2*), Chandler (*3*), Harvey (*5*), Rosa (*22*), Akerman (*1*), Maximov (*12*), Steinmetz (*24*), and others, and therefore only the more pertinent papers will be mentioned in connection with the discussion of each environmental factor.

MATERIALS AND METHODS

EQUIPMENT

All results herein reported were obtained from seeds sown and from plants grown, hardened, frozen, and thawed in the greenhouse. The equipment used in the plant pathology greenhouses of the Nebraska Agricultural Experiment Station has been described in detail by Peltier (*17*). Briefly, the greenhouse, besides having the ordinary warm space maintained at a temperature of approximately 20° C., is equipped with a hardening room, which is cooled by air drawn over artificially refrigerated coils. The hardening room is similar to the warm greenhouse except that it has double-glass top and sides, with a 2-inch air space between the glasses. It can be maintained at a temperature varying between 0° and 5°.

Six temperature cases described by Peltier and Goss (*18*) were placed at one side of the hardening room. These were electrically heated and could be maintained fairly constant at any desired temperature above the room temperature. The variation in temperature in these cases was less than ± 1° C. A current of air was forced through the cases constantly by means of an electric blower connected through a common conductor to each case. This blower had a capacity of 225 cubic feet per minute, and since the volume of all the cases was only 252 cubic feet (42 cubic feet each) there was a change of air approximately every 1⅛ minutes. Actually the change may not have been so rapid, since there was not a free movement of air through the cases. However, sufficient air passed through to keep the temperature constant. Two and one-half feet above one case was hung a 1,000-watt electric-light bulb, used for the long-day experiments. For the 17-hour day, the light was put on at 4 p. m. and was automatically switched off at the desired time by means of an alarm clock, the alarm winder of which was tied to a switch. No artificial light was used in the morning, so the light went off regularly at 10 to 12 o'clock midnight, as desired. In addition to the six cases mentioned, three cases at approximately 20° C. were placed in an adjoining warm room. These cases were used in preference to the ordinary greenhouse, as conditions in them were more nearly comparable to conditions in the cases in the hardening room.

The head house was equipped with a freezing room 8 by 7 by 8 feet, which could be maintained by artificial cooling at a temperature as low as −35° C. with a variation of ±2°.

The containers in which the plants were grown were of three types: Wooden flats, 15 by 18 by 7 inches; metal water-tight containers, 4 by 4 inches by 6 inches deep; and ordinary porous clay pots, 4 inches

in diameter. The seed was sown in rows in the flats, each flat containing 5, 6, or 7 rows with approximately 30 plants per row. The seed was sown broadcast in the pots and tin containers, each containing from 10 to 20 plants. No effort was made to have the number constant, as preliminary experiments indicated that a slight difference in the number did not introduce an appreciable error. Difficulties due to damping off of young plants during the short days in winter were largely overcome by wetting the soil in the pots thoroughly before planting, covering the seed with a shallow layer of sand, and supplementing daylight with powerful electric lamps for a portion of the night.

The soil used throughout the experiments was a rich loam mixed with sand in the proportion 4:1.

VARIETIES USED

In practically all the work three to six varieties of alfalfa were used, including the following: Turkestan, F. C. I.[5] No. 15754; Grimm, F. C. I. No. 15713; Nebraska common; Utah common, F. C. I. No. 15815; Arizona common, F. C. I. No. 15837; and Ladak, F. C. I. No. 14135. If only three were included, they were usually Turkestan, Grimm, and Arizona common. In a few of the later experiments Grimm alone was used. The first year the seed was treated with concentrated sulphuric acid for 10 minutes before it was sown. This was to insure against "hard" seeds coming up after freezing. With more experience it was found that new seedlings could readily be distinguished, and, except in a few instances, treating was discontinued the second year.

MEASUREMENT OF HARDENING

The response of a plant to the hardening process was measured by its ability to survive cold in the artificial freezing chamber. Except where specially noted, the technic followed in all the experiments herein reported, which in all instances was based on previous experiments, has been described by Peltier and Tysdal (*20*). The main features common to all tests were as follows: 12 to 16 hours before exposure in the freezing room the porous pots or flats were saturated with water. Natural drainage left the soil in different pots or flats at approximately the same moisture content. During freezing the temperature in the freezing room was maintained as nearly uniform as possible. Relatively short exposures at rather low temperatures were employed. Immediately after freezing, the plants were transferred to a warm room, where they were allowed to recover.

In all experiments the measure of survival of the plants was based on actual counts. The plants were counted before they were put into the hardening room and again two weeks after they were frozen. From these two numbers the percentage survival was calculated. In practically all the experiments the freezing was so severe that the tops of the plants were completely killed, and the surviving plants had, therefore, to start new growth from the crown. These plants showed a great difference in the rate of recovery, depending on the degree of injury. An estimate of the degree of injury might therefore

[5] The letters F. C. I. indicate accession numbers of the Division of Forage Crops and Diseases.

have been used as a basis for tabulating differences. This method has been used by Hill and Salmon (*7*), Quisenberry (*21*), and others, but since it is subject to errors of judgment, it was considered more accurate, in the case of alfalfa at least, to make an actual count of the plants surviving.

AGE OF PLANTS AT FREEZING

Steinbauer (*23*) found red-clover plants to be least resistant to cold at 21 days of age, when they were forming the first pair of permanent leaves. In alfalfa, however, Peltier and Tysdal (*20*) found that resistance began earlier and increased with age up to 60 days, except that seedlings 5 days old, just emerging from the soil, were more resistant than those 10 days old with two cotyledons and the third leaf showing. The relative varietal response at 25 to 30 days was the same as at 53 or 60 days. As a result of these findings, the plants used in the following studies were allowed to grow in the warm greenhouse approximately 30 days, and unless otherwise stated this procedure was followed throughout.

LENGTH OF TIME IN HARDENING ROOM

Peltier and Tysdal (*20*), in studying the influence of length of the hardening period on resistance to cold, kept alfalfa plants in the hardening room at temperatures of 2° to 4° C. from 1 to 35 days. They found that resistance increased with increasing time in the hardening room up to 14 to 15 days. Thereafter it remained much the same up to 23 days; at 35 days it was again low, probably due to exhaustion of the plants. This decrease in resistance corresponds with observations by Molisch (*13*), who found that plants continuously exposed to a temperature too low for normal metabolism, but above the freezing point, die. From the results of these experiments it was concluded that a 14-day period would be optimum for hardening under constant conditions. It is realized that this period may not be best under all conditions, but it affords a basis for standardizing the experiments in this respect, without which it would be impossible to deal satisfactorily with the large number of variables involved.

INFLUENCE OF LIGHT ON THE HARDENING PROCESS

To determine the effect of light on the hardening process in the different alfalfas, three groups of experiments were made, namely, those primarily concerned with (1) the influence of length of day, (2) the influence of light intensity, and (3) the influence of wave length of light.

INFLUENCE OF LENGTH OF DAY

In general, the results obtained by investigators of the relation of light to plant growth point to decreased growth and increased chlorophyll concentration with decreased light intensity. Low light intensities favor top growth at the expense of root growth, although on this point there is some difference of opinion. Greater storage, or at least greater manufacture, of carbohydrates takes place in the long-day plants. Small variations in the quality of light do not markedly affect plant growth, but optimum growth occurs under a complete spectrum rather than under a portion of it. Length of day exhibits a more striking influence on development of plants than does intensity of light.

Oakley and Westover (*16*) clearly demonstrated that the prostrate habit exhibited by some species and varieties of alfalfa under shortened day length in the fall might be traced to their response to light. However, they did not show whether this response to light actually served to harden the plant.

The present study was undertaken to determine the part played by length of day in the hardening process in alfalfa varieties. In fact, this work was started with the assumption that a long day would build up carbohydrate reserves in the plant, and results reported by Graber et al. (*4*), Willard (*25*), and others indicated that large carbohydrate reserves better enable plants to overwinter successfully. From this it appeared that the longer the day, within certain limits, the better chance the plant would have of surviving low temperatures. As will be seen, this hypothesis proved to be incorrect.

The experiments to determine the influence of light on the hardening process were made in the cases in the hardening room and adjoining warm room. These cases were large enough to contain thirty-two 4-inch clay pots. Rubberized light-proof hoods were made to fit over the individual cases and were used to exclude light from those in which the short-day plants were placed, while artificial light from a 1,000-watt Mazda C lamp placed 2 feet above the case was used to supplement ordinary daylight for the long-day plants. The cases could be maintained at any temperature above the hardening-room temperature of about 5° C. They were aerated by a continuous stream of air from a blower connected through a common duct to all cases.

The experiments generally were planned so that three cases were regulated to as nearly the same temperature as possible, and the length of day varied between these three cases. In only two experiments did the average temperature vary more than 0. 5° C. between different cases, and in these two experiments only slightly more than 0.5°. The 17-hour day was made up of the normal light plus sufficient artificial light to make up 17 hours of continuous light. In practically all the experiments both a day of normal length for the particular period of the year and a 17-hour day were used for comparison with a 7-hour day. The 7-hour day, unless otherwise specified, extended from 9 a. m. to 4 p. m.

A large number of experiments were run at the two temperatures 20° and 11° C. This was done for the purpose of determining beyond a question of a doubt the performance of these alfalfa varieties as regards cold resistance under this particular set of conditions. Early in the work, in fact as soon as the first experiment was completed, it was found that there was a decided difference in response at the two temperatures. The difference was so striking that the results probably could have been taken as conclusive, but they were so surprising that additional data seemed necessary to substantiate them. This was done by running several more sets under much the same conditions. To avoid the possibility of exposing the plants in the short-day case to the same environmental conditions and thus reflecting the same results in the following experiments, the short-day and normal-day cases were interchanged, one being used for the short day in one experiment and for the normal day in the following experiment. This could not be done, nor did it seem essential, with the 17-hour-day case. The light from the lamp used for artificial illumination was

kept from scattering to adjacent cases by curtains which were drawn each afternoon around the 17-hour case.

The plants were kept under these special light and temperature conditions for two weeks. No attempt was made to study the time factor in connection with the influence of light on the hardening process. While it is recognized that this factor may be important, it was considered impossible adequately to carry through such a study at the time these experiments were made. The multiplicity of combinations involved in using several different varieties at different day lengths, at different temperatures, and for different lengths of time necessarily precluded as thorough a study as might have been desired. Since the 2-week period proved successful in the temperature study, and since it gave striking differences in the light study, it was considered that the underlying principle of light response might be more or less accurately determined. In all, 25 experiments were made to determine the influence of light on the hardening process. These involved the use of 1,370 porous clay pots, with a total of approximately

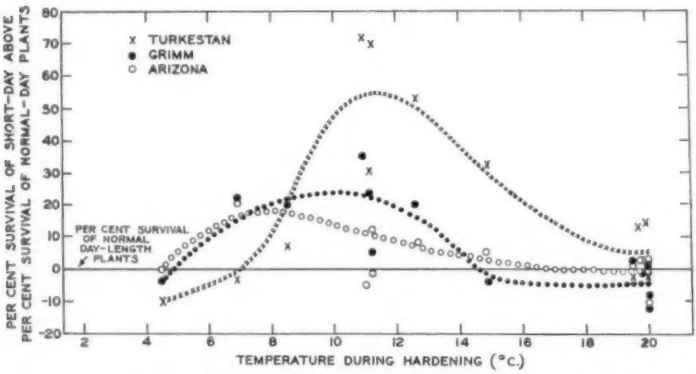

FIGURE 1.—Influence of short (7-hour) day, as compared with that of the normal day, on resistance to cold of three alfalfa varieties hardened at various temperatures

16,000 plants, assuming an average of 12 plants to the pot. The results of the uniform short-day, normal-day, and long-day experiments from which the varietal responses are calculated are given in Table 1.

In the original plan two temperatures were included, namely, approximately 10° and 20° C. The first results showed that there was a difference in response to length of day at the two temperatures. Thus, the whole question of the interrelation of light and temperature was apparently involved. Although the work has not been completed as fully as might be desired, it is believed that the results presented at this time are sufficiently consistent and include a sufficiently wide range of temperatures to indicate the principles involved.

For purposes of discussion the influence of length of day on the hardening process of the several varieties will be considered chiefly at three temperatures, viz, 20°, 11°, and below 10° C. Figure 1, based on Table 1, shows graphically the response of the three varieties to light at the different temperatures. The location of the dots is

TABLE 1.—*Influence of length of day on ability of alfalfas to resist cold*

Experiment No.	Hardening temperature	Length of normal day during the hardening period	Freezing exposure			Replications of each variety	Survival of plants frozen after 2 weeks' exposure to indicated day length									Probable error of the mean [a]
			Date	Duration	Temperature		Turkestan			Grimm			Arizona common			
							7 hours	Normal day	17 hours	7 hours	Normal day	17 hours	7 hours	Normal day	17 hours	
	°C.	*H. m.*		*H. m.*	°C.	*Number*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
21	25.0	12 42	Apr. 8, 1931	7 0	−13.0	8	43	97	.....	.....	.....	.....	6	64	.....	5.0
9a	20.0	10 16	Dec. 13, 1930	3 0	−14.0	5	65	67	82	64	76	80	31	28	27	5.5
2a	20.0	10 10	Nov. 19, 1929	4 15	−12.4	5	7	6	37	5	13	16	4	15	67	4.9
3a	19.8	9 37	Dec. 4, 1929	4 0	−12.9	5	14	0	20	0	0	2	3	0	2	4.9
6a	19.7	9 36	Jan. 28, 1930	3 0	−14.2	5	16	3	13	5	6	2	4	1	11	3.9
1a	19.5	10 37	Nov. 4, 1929	4 35	−12.4	5	0	2	4	2	0	0	1	0	4	3.8
Average	.....	.....	.....	.....	.....	.....	20	16	31	15	19	20	9	9	22	.....
15	14.8	9 56	Nov. 25, 1930	4 40	−14.6	8	49	18	44	13	17	29	19	14	19	4.8
1b	12.6	10 37	Nov. 4, 1929	4 35	−12.4	5	58	6	5	26	6	8	7	0	0	3.8
19	12.0	9 48	Jan. 30, 1931	5 0	−13.9	8	74	.....	32	.....	.....	.....	31	.....	10	5.4
9b	11.2	10 16	Feb. 13, 1930	4 30	−14.2	5	.....	.....	.....	.....	.....	.....	[b] 44	[b] 45	[b] 49	5.5
2b	11.2	10 10	Nov. 19, 1929	5 50	−13.0	5	88	20	49	18	13	23	30	18	6	4.9
6b	11.1	9 36	Jan. 28, 1930	5 0	−14.1	5	54	24	24	27	4	12	4	9	4	3.9
3b	10.9	9 37	Dec. 4, 1929	5 30	−13.0	5	71	0	35	35	0	5	9	0	5	4.9
Average	.....	.....	.....	.....	.....	.....	71	15	36	27	5	13	14	9	5	.....
14	8.5	13 45	May 3, 1930	6 30	−14.7	13	81	74	.....	45	25	.....	.....	.....	.....	5.8
20	6.9	10 27	Feb. 17, 1931	4 45	−13.0	8	71	74	67	69	47	54	46	25	29	7.1
8	4.5	10 15	Feb. 12, 1930	5 0	−14.2	5	84	94	.....	87	91	.....	54	54	.....	5.8
22	4.3	13 42	May 5, 1931	6 40	−13.3	16	94	76	.....	.....	.....	.....	66	50	.....	5.7

[a] Calculated by the deviation-from-the-mean method (*6a*).
[b] Not included in averages.

determined for each variety by the amount by which that variety under the 7-hour day exceeds or falls short of the percentage survival of the normal-day plants at the same temperature. The normal-day plants hereafter will be referred to as checks. It should be remembered that the plants were handled similarly throughout each experiment except in the matter of variation in length of day.

At 20° C. none of the varieties under the short day differed greatly in percentage of survival from the checks grown under normal day. In no instance was the deviation from normal-day survival greater than 15 per cent. As compared to the check, Turkestan averaged slightly higher, Grimm about the same, and Arizona common slightly less. At 11°, however, the results were strikingly different. At this temperature Turkestan hardened decidedly more under the short-day

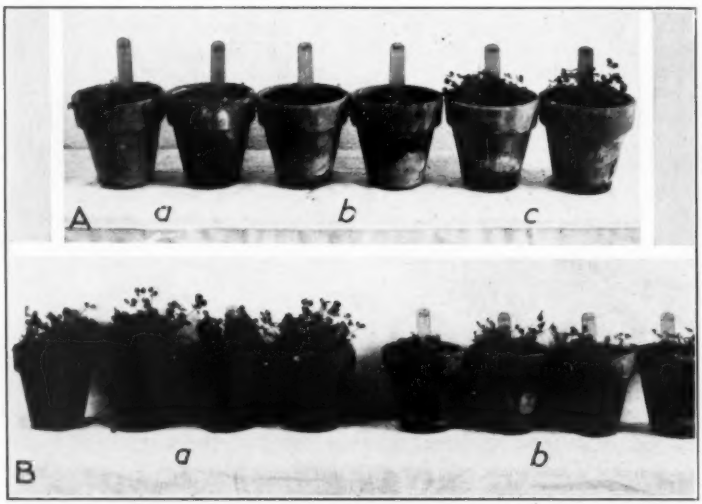

FIGURE 2.—Influence of length of day on the ability of alfalfas to resist cold: A, Plants of Turkestan variety hardened at 12° C.; *a*, 2 pots of plants hardened under a day length of 10 hours and 10 minutes; *b*, 2 pots of plants hardened under a day length of 17 hours; *c*, 2 pots of plants hardened under a day length of 7 hours. Photographed 14 days after freezing. B, Plants of Grimm variety hardened at 11.5°; *a*, 4 pots of plants hardened under a day length of 7 hours; *b*, 4 pots of plants hardened under a day length of 11 hours and 54 minutes. Photographed 30 days after freezing

than under the normal-day length. The average survival of the short-day plants was 71 per cent, while that of the normal-day plants was only 15 per cent. Grimm responded to the short day to a less extent than Turkestan, but considerably more than Arizona common.

Averaging the three tests conducted at approximately 11° C., in which all varieties are represented, it is found that the difference in percentage of survival in favor of the short-day plants over the normal-day plants is, for Turkestan, 56; for Grimm, 22; and for Arizona common, 5. Photographs of the plants two to four weeks after freezing are shown in Figure 2.

One experiment conducted at 25° C., when the normal length of day was 12 hours and 42 minutes, shows clearly that the 7-hour day is detrimental to the plants at this temperature and that they are not able to survive cold as well as the normal-day plants. (Table 1.)

This was the only experiment in which the short-day plants showed even a slight yellowing.

At temperatures between 10° and 20° C. the response of the varieties is found to be more or less intermediate. Turkestan apparently begins the hardening process in response to short day at a higher temperature and to a greater extent than the other two varieties. At temperatures below 10° the influence of light apparently diminishes rapidly, and at 4° there is practically no difference in survival between the short-day and the normal-day plants of any of the varieties.

If the normal-day plants are compared with the long-day plants it is found that on the average the percentage of survival is slightly higher in the long-day plants both at 10° and at 20° C. Again the response is greater in Turkestan than in either of the other varieties. At lower temperatures the long-day plants have practically the same survival as the normal-day plants in all varieties.

The increased survival of the long-day plants at the higher temperature may be explained in part by the greater growth that took place under these conditions. It has been shown that increased size, within certain limits, enables a plant better to withstand cold. The increased growth of plants under the long day is shown in Table 2, and also in Figure 3. Turkestan again is more responsive to the influence of length of day than either of the other varieties.

The Ladak variety was used in several of the later experiments because it was known that this variety begins dormancy very early in the fall. In these tests short day length influenced the hardening process in Ladak slightly more than in Turkestan, indicating a close correlation between these results and field behavior.

In three of the length-of-day experiments carried out at 11.5° and at 20° C. the height of the plants was measured just before they were put in the hardening chamber and again just before they were frozen. The actual change in the height of the plants during this 2-week period is shown in Table 2. At the lower temperature and short day, Turkestan grew 0.2 cm in two weeks and Arizona 1.4 cm. The growth of Grimm was intermediate. At the higher temperature and short day, Turkestan grew 1.9 cm and Arizona 3.2 cm. However, with a long day Turkestan grew almost as much as Arizona at both the lower and higher temperatures. These results agree with those of Oakley and Westover (*16*).

TABLE 2.—*Growth of plants for a 2-week period under controlled conditions of light and temperature*

[Average of three experiments]

Length of day	Temperature	Growth of plants during the 2-week period			Length of day	Temperature	Growth of plants during the 2-week period		
		Turkestan	Grimm	Arizona common			Turkestan	Grimm	Arizona common
H. m.	*°C.*	*Cm*	*Cm*	*Cm*	*H. m.*	*°C.*	*Cm*	*Cm*	*Cm*
7 0	20	1.9	1.9	3.2	7 0	11.5	0.2	0.7	1.4
10 30	20	4.3	3.9	5.3	10 30	11.5	1.5	1.8	2.3
17 0	20	5.4	4.5	6.1	17 0	11.5	3.1	3.2	3.8

The growth changes at 11° C. are in close agreement with the actual survival tests from freezing; the fact that this can not be said of the results at 20° indicates that slowing up of growth does not necessarily mean hardening of the plant.

In view of the decided hardening response of plants to the 7-hour day, further information was desired on the influence of a still shorter day, and on the length of day at which plants become weakened from lack of light. Accordingly, in December, 1929, experiments were made in which a 3-hour and a 6-hour day were used. After the

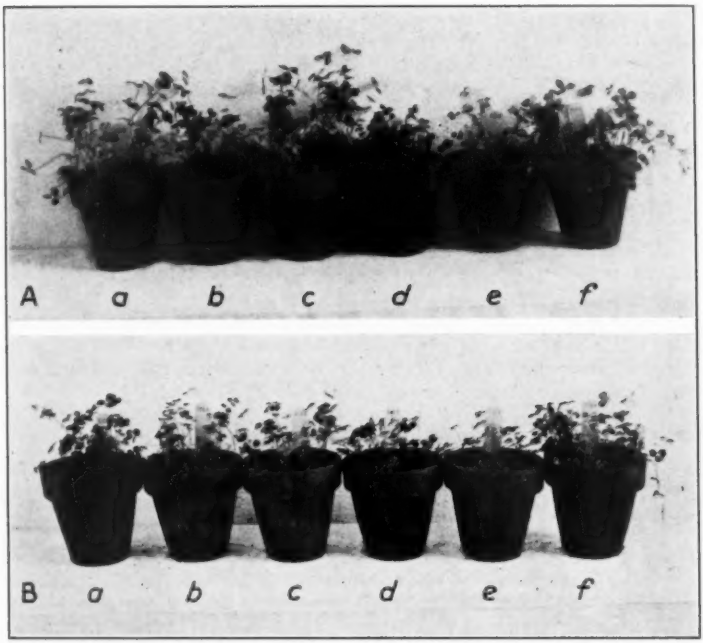

FIGURE 3.—Alfalfa varieties hardened under different day lengths and grown at different temperatures; photographed after 11 days under specified conditions and before freezing. A, Plants hardened at 20°C.: *a*, *b*, and *c*, Turkestan, Grimm, and Arizona varieties, under 17-hour day; *d*, *e*, and *f*, same varieties, under 7-hour day. B, Plants hardened at 12°: *a*, *b*, and *c*, Turkestan, Grimm, and Arizona varieties, under 17-hour day; *d*, *e*, and *f*, same varieties, under 7-hour day. Note greater growth at higher temperature and under longer day. Under short day Arizona produces more growth than either Turkestan or Grimm

plants had been kept under these short-day conditions they were frozen in the artificial freezing chamber and counts made of those that survived.

The results (Table 3) indicate that the 3-hour day was very detrimental to the plants. Even among the 6-hour-day plants the number surviving was smaller than among the normal-day plants. Experiments conducted later in the year (March) indicated that a 5-hour day might not be too short for optimum survival.

INTERRELATION BETWEEN INFLUENCE OF LIGHT INTENSITY AND OF DAY LENGTH

During the course of the experiments on the influence of length of day on the hardening process, tests including the 7-hour day were made throughout the winter of 1930–31. It was surprising to find that during certain portions of the year even the 7-hour day did not give the high percentage of survival that it ordinarily did. The results obtained in all these studies seem to point to the fact that intensity of light as well as length of day has an important bearing on the problem. For purposes of comparison most of the experiments carried out during the period of low light intensity in the winter, together with the special experiments involving shorter day lengths, are brought together in Table 3.

TABLE 3.—*Interrelations between the influence of light intensity and that of day length on the ability of alfalfas to resist cold*

Experiment No.	Date of freezing	Total solar radiation (average per day)		Length of day	Hardening temperature	Freezing exposure		Replications of each variety	Survival				Probable error of the mean
		Outside	In hardening room			Duration	Temperature		Turkestan	Grimm	Arizona common	Ladak	
		Gram calories per cm²	*Gram calories per cm²*	*H. m.*	*°C.*	*H. m.*	*°C.*	*Number*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
17	Dec. 26, 1930	140.5	49.9	7 00	7.5	7 35	−14.1	8	0	0	0	0	
		145.3	51.6	9 15	6.9	7 35	−14.1	8	13	0	0	0	3.2
				17 0	7.0	7 35	−14.1	8	1	0	0	0	
16	Dec. 12, 1930	138.9	49.3	7 0	12.0	6 0	−14.6	8	35	17	14	32	5.8
		149.3	53.0	9 24	11.9	6 0	−14.6	8	23	6	0	4	
				17 0	11.6	6 0	−14.6	8	30	8	9	11	
16b	do	138.9	49.3	7 0	14.8	5 0	−14.0	8	23	18	13	25	5.8
		149.3	53.0	9 24	14.9	5 0	−14.0	8	27	40	8	5	
				17 0	14.7	5 0	−14.0	8	22	16	4	6	
4	Jan. 4, 1930	73.2	26.0	3 0	2.9	5 0	−13.6	6	5	12	0		6.2
		169.9	60.3	6 0	3.0	5 0	−13.6	6	34	26	2		
		197.6	70.1	9 15	3.0	5 0	−13.6	6	40	52	30		
18	Jan. 15, 1931	183.2	65.0	7 0	10.8	5 35	−13.2	16	65		9		3.1
		197.8	70.2	9 27	10.5	5 35	−13.2	16	38		2		
				17 0	10.5	5 35	−13.2	16	14		2		
8	Feb. 12, 1930	164.0	58.2	5 0	4.6	5 0	−14.2	5	94	64	51		5.8
		220.2	78.2	7 0	4.4	5 0	−14.2	5	84	87	54		
		242.4	86.1	10 15	4.6	5 0	−14.2	5	94	91	54		
5	Jan. 27, 1930	92.6	32.9	3 0	4.4	4 15	−14.5	5	23	39	36		7.2
		193.2	68.6	6 0	4.2	4 15	−14.5	5	64	57	54		
		245.1	87.0	9 36	4.6	4 15	−14.5	5	76	70	41		
14	May 3, 1930	208.9	74.2	5 0	8.5	6 30	−14.7	13	82	44			5.8
		281.5	99.9	7 0	8.0	6 30	−14.7	13	81	45			
		375.8	133.4	13 45	8.9	6 30	−14.7	13	74	25			
11	Mar. 22, 1930	252.8	89.7	5 0	11.8	6 30	−14.5	8	97	73	26		6.8
		337.2	119.7	7 0	11.5	6 30	−14.5	8	85	85	28		
		391.4	138.9	11 54	11.4	6 30	−14.5	8	76	54	27		

Since no apparatus for measuring light in the greenhouse was available, the radiation intensities were obtained from continuous records kept at this station by the United States Weather Bureau, by averaging the total for the given number of hours per day for the period the plants were under the special conditions. For example, if the plants were under a 5-hour day, the total radiation for those 5 hours is given. As the observations were taken on sun time they had to be corrected to standard time. A further correction was necessary because of the fact that the observations were taken in the open air,

whereas the plants were grown under four thicknesses of glass. This correction was made by the use of a correction factor worked out by placing four thicknesses of the type of glass used in the greenhouse over the pyrheliometer. Measurements with the pyrheliometer indicated that four thicknesses of glass cut down the total solar radiation to 37.9 per cent of normal when the glass was perpendicular to the rays of the sun, and to 33.2 per cent when the glass was at an angle of 45°. Having an air space between the glasses did not appreciably affect the reduction in solar radiation. Since the angle of the sun varies, an average figure of 35.55 was taken, and in Table 3 the solar radiation in the hardening room is calculated as 35.55 per cent that of the radiation outside. Thus, while the figures on light intensity given in Tables 3 and 4 were not taken in the greenhouse where the plants were growing, they should be approximately correct.

In Table 3 at least two, and usually three, lengths of day are given for each experiment. In all experiments the percentage survival of the plants under normal day length is used as the standard of comparison.

A summary of Table 3 is presented in Table 4, where the survivals are arranged in the order of intensity of light.

The percentage survival of the short-day plants is compared with the percentage survival of the normal-day plants, grown and frozen under the same conditions. In analyzing the results it is necessary to consider the different temperatures in the hardening chamber separately. The two main temperatures involved are 3° to 4.5° and about 11° C.

At 4° the normal-day plants are higher in survival in all experiments than the short-day plants regardless of the light intensity, but there is a greater difference in the spread at the lower intensities than at the higher. Judging by the low percentage of survival, the 3-hour day, having a total solar radiation in the hardening chamber of 26 to 32.9 gram calories per square centimeter, is obviously too short or gives insufficient light. At higher intensities and longer day, the survival of the short-day plants approaches that of the normal-day plants.

Experiment 14 of Table 3 shows that a 5-hour day may be sufficiently long if the light intensity is great enough.

At 11° C. the average survival, without exception, is in favor of the short day, in accordance with the decided response at this temperature, and this in spite of the fact that the light intensity is very low for some of the experiments. It should be noted that no 3-hour days were tested at this temperature, the shortest day being five hours.

The relationship between solar-radiation intensity and daylight illumination has been worked out by Kimball (*9*), who found a factor of 6,700 for the ratio

$$\frac{\text{Illumination intensity (foot-candles)}}{\text{Radiation intensity (gram calories per minute per cm}^2\text{)}}.$$

He states that this will give the daylight intensity within about ±5 per cent. To use this formula on the radiation data given in Tables 3 and 4 it is necessary to divide the gram calories per square centimeter per day by the number of minutes for that particular length of day.

TABLE 4.—*Summary of interrelations between the influence of light intensity and that of day length on the ability of alfalfas to resist cold*

Total solar radiation (average per day)		Hardening temperature	Length of short day	Average survival of three varieties under [a]—		Total solar radiation (average per day)		Hardening temperature	Length of short day	Average survival of three varieties under [a]—	
Outside	In hardening room			Short day	Normal day [b]	Outside	In hardening room			Short day	Normal day [b]
G cal. per cm²	*G cal. per cm²*	*°C.*	*Hrs.*	*Per cent*	*Per cent*	*G cal. per cm²*	*G cal. per cm²*	*°C.*	*Hrs.*	*Per cent*	*Per cent*
73.2	26.0	3.0	3	5.7	40.7	208.9	74.2	8.5	5	[d] 63.0	[d] 49.5
92.6	32.9	4.4	3	32.7	62.3	281.5	99.9	8.5	7	[d] 63.0	[d] 49.5
164.0	58.2	4.5	5	69.7	79.7	138.9	49.3	11.8	7	22.0	9.7
169.9	60.3	3.0	6	20.7	40.7	183.2	65.0	10.6	7	[c] 37.0	[c] 20.0
193.2	68.6	4.4	6	58.3	62.3	252.8	89.7	11.6	5	65.3	52.3
220.2	78.2	4.5	7	75.0	79.7	337.2	119.7	11.6	7	66.0	52.3
140.5	49.9	7.1	7	0	[c] 13.0	138.9	49.3	14.8	7	18.0	25.0

[a] Unless otherwise shown, averages are for three varieties—Turkestan, Grimm, and Arizona.
[b] Normal day ranged from 9¼ hours to 13¾ hours, the total solar radiation of course being greater than that shown for the short day in the first 2 columns.
[c] 1 variety, Turkestan.
[d] 2 varieties, Turkestan and Grimm.
[e] 2 varieties, Turkestan and Arizona.

INFLUENCE OF WAVE LENGTH OF LIGHT

To determine the influence of the wave length of light on the hardening process, three cages 15 inches high, 24 inches wide, and 36 inches long were constructed of transparent cellophane. The top and sides were covered with the material, but the bottom was left open, thus allowing free circulation of air when the cages were placed on a slat bench. One cage was red; another white; and the third, blue. They were placed over the pots containing the plants in the hardening room at a temperature of approximately 3.5° C. Spectrophotometric measurements of the transmissibility of radiation by the three colors of cellophane used are given in Figure 4. The transmissibility of radiation by the clear cellophane was high at all wave lengths measured, from the blue to the red. Unfortunately, measurements could not be made of wave lengths lower than 480 mμ. The transmissibility of radiation by red cellophane was very low in the blue and the green, but increased rapidly in the yellow, the orange, and the red. The blue cellophane transmitted a high percentage of the blue, lower percentages of the green, and very little of the yellow, orange, or red. In general, therefore, the plants under the red cellophane received light from the red end of the spectrum, the plants under the blue cellophane received light from the blue end of the spectrum, and those under the clear cellophane received white light. The last mentioned was included to serve as a check.

Two separate series were run under these cages, with three different varieties, Turkestan, Grimm, and Arizona common. The results of the two series combined are presented in Table 5. In both series the plants under the white cage proved to be the most resistant to artificial freezing. The red and blue lights produced practically the same degree of hardening, the red averaging slightly less.

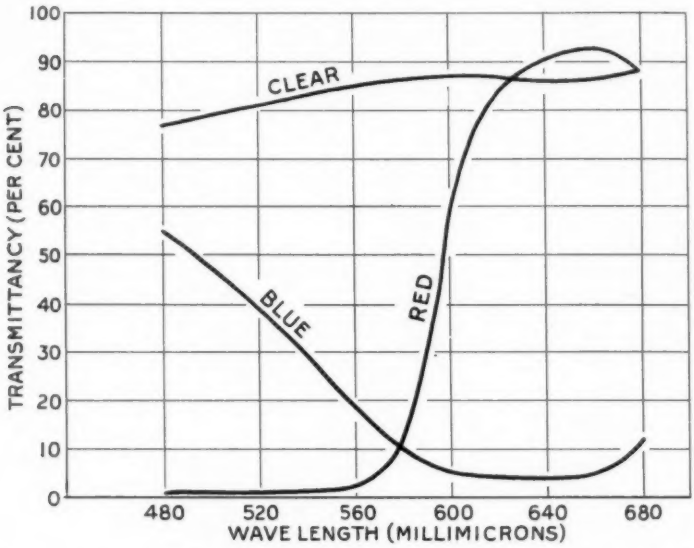

FIGURE 4.—Transmissibility of radiation by the three types of cellophane used in experiments to determine the influence of different wave lengths of light on the hardening process

TABLE 5.—*Influence of light of different wave lengths on ability of alfalfas to resist cold*

Light	Survival			Average of all varieties
	Turkestan	Grimm	Arizona common	
	Per cent	*Per cent*	*Per cent*	*Per cent*
Red	63	68	32	54
Blue	60	65	50	58
White	77	81	44	67

INFLUENCE OF TEMPERATURE ON THE HARDENING PROCESS

It has generally been conceded that temperature is one of the major factors in the hardening process, and hardening is usually associated with lower temperatures. However, it has been known for some time that plants can be hardened by other means than by lowering the temperature. Rosa (*22*) hardened cabbage plants chiefly by four methods: (1) Exposing them in an open frame to temperatures near freezing during the night and to full sunlight during the day; (2) giving them just enough water to keep them growing slowly (this lot usually showed the maximum degree of hardening in the cold frame); (3) pruning the roots severely by running a knife close to the stem on one or both sides of the plant (this treatment checked the growth of the plants materially for a short time and somewhat increased their resistance to cold); (4) by watering with N/10 solutions of various salts.

In Rosa's work (*22*) the degree of hardening was determined by the ability of the plants to withstand cold. He states that the growth of

plants subjected to any of the hardening treatments was checked in proportion to the intensity of the treatment and that any treatment that materially checks the growth of plants increases their resistance to cold. He used cabbage chiefly in his work, and his freezing exposures were necessarily not severe.

Harvey (*6*) has shown a correlation between hardening temperature and cold resistance. He states that the "threshold value" for the hardening process is approximately 5° C. for cabbages. He also suggests that alternating temperatures harden cabbages and that 1 hour's exposure to cold in every 24 hours has a hardening effect.

While the importance of temperature in the hardening process is more or less taken for granted, very few actual data except those confined to horticultural crops can be found which show the relation of hardening temperatures to ability to withstand cold. Having this in mind, and also the ever-present problem of causing as wide differences between hardy and nonhardy varieties as possible, the writer made several experiments under controlled temperature conditions, to determine the relation of hardening temperatures to cold resistance.

INFLUENCE OF VARIOUS CONSTANT TEMPERATURES

The cases in the hardening room could be held at any desired temperature above that of the hardening room with a variability of about 1° C. During the season 1930–31 two cases were fitted with blowers which drew cold air from around artificially refrigerated coils, discharging this air directly into the case. By this means these cases could be maintained at a relatively constant temperature below that of the hardening room and as low as −5° C.

Altogether, 10 experiments were carried out in which temperatures ranging from −5° to 30° C. were used. The plants remained under these special hardening conditions for 14 days. The object in using such a wide range of temperatures was to determine at what temperature hardening begins, when it reaches a maximum, and how low it may be before complete killing occurs. Other factors studied involved varietal response and the determination of the portion of the temperature scale at which hardening progresses most rapidly for each change of 1 degree in temperature.

The results of these experiments, presented in Table 6, show that within certain ranges the lower the hardening temperature the greater is the ability of the plants to resist cold. Experiments 1, 2, and 3, with temperatures ranging from 2° to 20° C., show a progressively higher percentage of survival at each progressively lower temperature. In fact, in experiments 2 and 3, the plants hardened at the higher temperatures were not exposed so long to freezing as those hardened at the lower temperatures, for if frozen at the same temperature the former would be completely killed, while the latter would show very little killing.

To determine the highest temperature at which the hardening process begins, experiments 4 and 5 were conducted. Temperatures of 10°, 15°, and 20° C. were used. The results show that a certain amount of hardening takes place at 15°. In experiment 6, in which temperatures of 15°, 20°, and 25° are compared, it is interesting to note that the plants hardened at 20° show a slightly greater resistance to cold than those hardened at 25°.

TABLE 6.—*Influence of temperature during hardening on the ability of alfalfas to resist cold*

Experiment No. and period [a]	Hardening temperature	Freezing exposure		Replication in each variety	Survival of—						Probable error of mean
		Duration	Temperature		Turkestan	Grimm	Arizona common	Nebraska common	Utah common	Ladak	
	°C	H. m.	°C	No.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1, Oct. 2 to Nov. 4, 1929	7.1	4 35	−12.4	6	96	75	41	98	71	.....	4.7
	8.0	4 35	−12.4	6	96	48	22	85	63	.....	4.7
	10.5	4 35	−12.4	6	60	20	3	24	11	.....	4.7
	12.3	4 35	−12.4	6	20	6	0	.....	.....	.....	4.7
	19.5	4 35	−12.4	6	4	0	0	.....	.....	.....	4.7
2, Oct. 8 to Nov. 19, 1929	3.6	5 50	−13.0	5	98	98	96	96	98	.....	4.3
	5.3	5 50	−13.0	5	93	97	61	92	89	.....	4.3
	9.1	5 50	−13.0	5	79	53	26	16	49	.....	4.3
	11.3	5 50	−13.0	5	18	13	18	6	20	.....	4.3
	19.9	4 15	−13.0	5	6	13	15	8	5	.....	4.3
3, Oct. 24 to Dec. 4, 1929	2.0	7 25	−13.0	5	77	59	34	37	75	.....	6.8
	4.7	7 25	−13.0	5	62	23	10	29	21	.....	6.8
	8.6	5 30	−13.0	5	18	7	0	1	0	.....	6.8
	10.9	5 30	−13.0	5	0	0	0	0	0	.....	6.8
	19.7	4 0	−13.0	5	0	0	0	0	0	.....	6.8
4, Oct. 10 to Nov. 25, 1930	9.9	3 30	−13.1	8	73	83	58	.....	.....	86	5.2
	14.3	3 30	−13.1	8	44	56	33	.....	.....	38	5.2
	20.2	3 30	−13.1	8	10	11	11	.....	.....	15	5.2
5, Oct. 27, to Dec. 11, 1930	10.3	4 0	−12.0	8	80	75	46	.....	.....	70	4.8
	14.9	4 0	−12.0	8	38	36	12	.....	.....	19	4.8
	20.0	4 0	−12.0	8	18	19	5	.....	.....	3	4.8
6, Nov. 11 to Dec. 27, 1930	15.0	3 30	−9.1	8	67	52	25	.....	.....	48	4.7
	20.4	3. 30	−9.1	8	31	12	4	.....	.....	14	4.7
	24.5	3 30	−9.1	8	18	6	0	.....	.....	12	4.7
7, Dec. 12, 1930, to Jan. 31, 1931	5.6	5 0	−13.6	16	89	.....	53	.....	.....	.....	13.2
	10.2	5 0	−13.6	16	80	.....	51	.....	.....	.....	13.2
	−2.5	5 0	−13.6	16	0	.....	0	.....	.....	.....	13.2
8, Dec. 31, 1930, to Feb. 16, 1931	20.2	2 0	−12.8	8	38	24	13	.....	.....	11	5.2
	24.1	2 0	−12.8	8	32	20	16	.....	.....	13	5.2
	29.9	2 0	−12.8	8	55	21	44	.....	.....	17	5.2
9, Sept. 9 to Oct. 22, 1931	0	8 45	−13.4	10	43	21	12	.....	.....	.....	5.5
	6.0	8 45	−13.4	10	68	25	0	.....	.....	.....	5.5
10, Sept. 19, to Nov. 7, 1931	0	5 45	−13.7	10	55	53	22	.....	.....	.....	4.4
	6.0	5 45	−13.7	10	80	84	61	.....	.....	.....	4.4

[a] Date of seeding to date of freezing.

The influence of hardening temperatures below 0° C. is shown in experiment 7. It was intended to keep the temperature in one of the experimental cases at −5°, but owing to the large number of warm, sunny days the average temperature for the 2-week period was −2.5°. The temperature rarely, if ever, rose above 0°. In this experiment only half of the pots were exposed to the freezing temperature shown in Table 6. The other half were taken directly from the hardening room to the warm greenhouse without exposure in the freezing room. None of these plants recovered. Thus it is clear that plants taken from a warm greenhouse and exposed directly to a temperature as low as −5° will not survive.

In experiment 8 the hardening temperatures were higher than in any of the other experiments; that is, 20°, 24°, and 30° C. The percentage survival at 20° is higher than at 24°, and the percentage survival at 30° is also higher than at 24°. Figure 5 shows the recovery of alfalfas exposed to freezing after being hardened at various temperatures.

The influence of a constant temperature very near 0° C. on the hardening process is shown in experiments 9 and 10. When the

average of both experiments is considered, for all three of the varieties tested, it is evident that plants hardened at 0° are not so resistant to cold as those hardened at 6°.

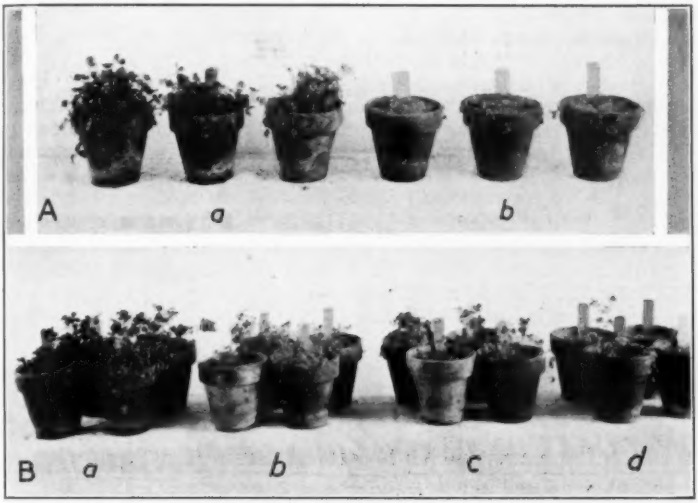

FIGURE 5.—Influence of temperature on the ability of alfalfas to resist cold: A, Grimm variety; *a*, 3 pots in hardening chamber at 4° C. two weeks before freezing; *b*, 3 pots not in hardening chamber before freezing. B, Turkestan variety, hardened for 14 days at various temperatures; *a*, 4 pots at 4°; *b*, 4 pots at 10°; *c*, 4 pots at 14°; *d*, 4 pots at 20°. All plants frozen for same length of time in artificial freezing chamber

Since the experiments were not conducted at the same time and since the freezing temperatures were necessarily different in different experiments, it is difficult to form a composite picture of the results

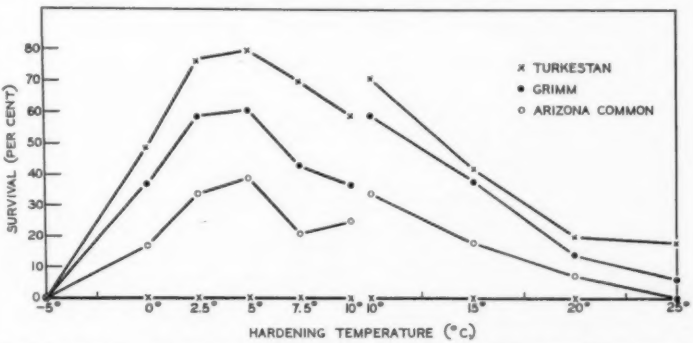

FIGURE 6.—Influence of hardening at different temperatures on the ability of three alfalfa varieties to resist cold

of the entire temperature range. Nevertheless, in Figure 6, an attempt has been made to combine the results of all experiments. The average percentage survival at each of the temperatures shown on the base line has been calculated from Table 6. The temperatures indicated

on the base line are taken as mid-points, and the average of all experiments with hardening temperatures nearest are included at these points. For example, all experiments having a hardening temperature between 12.5° and 17.5° are included in the average at 15° C. The figure has been divided into two parts, above 10° and below 10°, because those plants hardened at temperatures below 10° were as a rule frozen more severely than those hardened above 10°.

Figure 6 shows that plants hardened at 20° C. have a slightly higher percentage survival than those hardened at 25°. This difference, however, is not so marked as that between 15° and 20° or between 10° and 15°. The two hardy alfalfas, Turkestan and Grimm, show greater hardening at these temperatures than does the nonhardy Arizona common. The resistance of these varieties to cold increases when they are hardened at lower temperatures, maximum resistance being reached between 2.5° and 5°. When the plants are hardened at a constant temperature of 0° they show less hardiness than when hardened at 5°. No plants of any variety exposed to a temperature of −5° for a period of two weeks survived. If temperatures between −5° and 0° had been used it might have been found that all varieties were not killed at the same temperature.

Figure 6 shows that within certain limits there is a gradual increase in hardening as temperatures are lowered. These limits spread over a relatively wide range, from approximately 20° to near 0° C. There is no definite point at which hardening does or does not occur, the hardening process apparently being a gradual development. The amount of hardening for 1° change in temperature between 15° and 20° is not nearly so great as between 5° and 10°. The maximum rate of hardening for 1 degree change in temperature occurs at about 5°. Hardening of tender alfalfa plants at continuous temperatures much below 0° can not occur, for the plants are killed outright. Turkestan is more responsive to a change in temperature than Grimm, and Grimm than Arizona common. (Fig. 6.) This is directly correlated with the hardiness of the three varieties.

INFLUENCE OF ALTERNATING TEMPERATURES

In all the experiments thus far reported temperature and light were kept as uniform as possible. In the experiments now to be reported the hardening temperature was held constant for one set of plants, while duplicate sets were moved from one temperature chamber to another at intervals of 3 days. All plants remained in the hardening room for 12 days, and the set that was rotated went through four temperature changes. Thus, one set remained at 20° C. throughout the 12 days. A duplicate set was started at 15.4°, where it remained for 3 days; transferred to 11.4°, where it remained for 3 days; then to 5° for 3 days; and finally to 20° for 3 days, from which it was taken directly to the freezing chamber. In this experiment only one variety, Grimm, was used.

The results of two experiments have been combined and are presented in Table 7.

Table 7 shows that at 20° and 19.5° C. the percentage survival of plants from the alternating temperature was greater than that of plants from the continuous high temperature. Since the alternating-temperature plants had been exposed to a much lower temperature during the preceding three days, the increased survival may be attrib-

uted to a carry-over effect from the lower temperature. That this effect at the end of three days was not very great may be seen from the small differences recorded.

TABLE 7.—*Influence of alternating temperatures on the ability of Grimm alfalfa to resist cold*

Hardening temperatures (°C.)[a]	Percentage survival[b]	Hardening temperatures (°C.)[a]	Percentage survival[b]
15.4 to 11.4 to 5 to 20	2	5 to 20 to 15.4 to 11.4	29
20 continuous	0	11.4 continuous	34
14.3 to 10.8 to 3.5 to 19.5	14	3.5 to 19.5 to 14.3 to 10.8	14
19.5 continuous	10	10.8 continuous	62
11.4 to 5 to 20 to 15.4	11	20 to 15.4 to 11.4 to 5	0
15.4 continuous	0	5 continuous	19
10.8 to 3.5 to 19.5 to 14.3	6	19.5 to 14.3 to 10.8 to 3.5	0
14.3 continuous	24	3.5 continuous	14

[a] 3 days at each temperature.
[b] After freezing 7 hours at −15° C.

At 15.4° C. the behavior of the plants was much the same as at 20°, but at 14.3° a greater degree of hardening occurred than in those plants that passed through three days at higher temperatures. Moreover, from 11.4° to 3.5° the plants under the continuous temperature survived better than those that had been subjected to the high as well as the low temperatures, showing that a 3-day period at constant temperature did not harden the plants to the same degree as 12 days under the same conditions. Bearing in mind the influence of hardening temperatures on survival, it is interesting to note that increased survival of the plants under continuous treatment began at approximately 14°.

In order to study alternating temperatures on the basis of a 24-hour day, further experiments were conducted with the Turkestan and Arizona common varieties. In experiment 3, the results of which are given in Table 8, 16 pots of each variety were placed in a case held at an average temperature of 0.6° C. Each day eight pots of each variety were removed to a warm greenhouse (approximately 20°) and kept there for 8 hours (8 a. m. to 4 p. m.). The remainder of the pots were kept in the case. On the morning of the fifteenth day all the pots were transferred directly from the case to a freezing temperature of −13.2°, at which they were held for 5½ hours. The resulting survivals show that the plants subjected to alternating temperatures were able to resist the cold much more effectively than those held continuously at a low temperature.

A preliminary hypothesis, based on the results of experiment 3, assumed that the increase in hardiness of the alternating-temperature plants was due to the fact that at a high temperature the physicochemical processes stimulated by the low temperature could be carried on much more rapidly than in plants remaining at a temperature near 0° C. If this hypothesis is correct, alternating temperatures above a certain temperature should not give increased hardiness. This temperature would be the point at which the physicochemical processes could be carried on at as rapid a rate as the stimulus warranted, and no further increase in the rate of this reaction could be obtained by means of higher temperatures.

To test this hypothesis experiment 4 was conducted. Five different temperatures ranging from −5° to 11.3° C. were used. As in experiment 3, half of the plants were allowed to remain eight hours during daylight in the warm greenhouse. The results (Table 8) support the hypothesis.

TABLE 8.—*Influence of alternating temperatures on the ability of alfalfas to resist cold*

Experiment No. and period [a]	Hardening temperature	Freezing exposure		Replications of each variety	Survival after indicated conditions during hardening				Probable error of mean
		Duration	Temperature		Turkestan		Arizona common		
					Constant temperature	In warm greenhouse 8 hours each day	Constant temperature	In warm greenhouse 8 hours each day	
	° C.	H. m.	° C.	Number	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
3, Dec. 31, 1930, to Feb. 17, 1931	0.6	5 30	−13.2	8	44	87	8	87	6.9
4, Jan. 17 to Mar. 3, 1931	1.6	5 0	−13.6	8	40	74	14	77	6.9
	4.2	5 0	−13.6	8	69	88	79	75	6.9
	7.7	4 30	−13.2	8	92	92	76	55	6.9
	11.3	4 30	−13.2	8	88	76	47	51	6.9
5, Feb. 4 to Mar. 20, 1931	7.8	6 0	−13.0	8	84	98	72	65	5.6
	11.8	6 0	−13.0	8	77	39	36	49	5.6

[a] Date of seeding to date of freezing.

The −5° C. set is omitted from the table because at that temperature all the plants, even the alternating-temperature ones, were killed outright.

At a temperature of 1.6° C. the alternating-temperature plants were considerably higher in survival in both varieties. At 4.2° the Turkestan variety had a higher percentage survival at the alternating temperature, whereas Arizona common had a slightly lower survival. It is clear from these and later results that the hardy variety Turkestan must be treated separately from the unhardy variety Arizona common when speaking of their hardening response, since the former has a much greater capacity for hardening than the latter.

At 7.7° C. the percentage survival of Turkestan for both the continuous and alternating temperatures was the same, and at 11.3° there was a distinct reversal in favor of the continuous temperature, indicating that subjecting the plants to a still higher temperature for eight hours each day serves actually to lessen the amount of hardening. From these results it appears that the point at which the hardening activity is not greatly increased by higher temperatures is approximately 8° for the hardy variety and somewhat lower for the unhardy variety.

The relative increase in percentage survival of Arizona common at 11.3° C. may perhaps be explained by the added growth, as distinguished from hardening, which is possible at the higher temperature. This is not true of Turkestan, for it shows much more hardening at this temperature. (Fig. 6.)

The question arises as to the part played by light in the alternating-temperature effect. Since it has been demonstrated that light is an

important factor in the hardening process, might it not be true that through its influence on photosynthesis it has the ability to counterbalance the entire process of hardening?

To study this question and also to determine the length of time required at the low temperature for optimum stimulating effect on the hardening process, an experiment was conducted in which one hardening temperature, 3.8° C., and different alternating periods were used. In this experiment, reported in Table 9, one set of eight pots of each of the two varieties was kept in the hardening room (held at 3.8°) for a certain number of hours each day and then removed to the warm greenhouse (approximately 20°). In all, five sets of plants of each variety received this treatment. A sixth set remained in the hardening room continuously. Two additional sets were placed in two cases used in experiment 5, Table 8, and held at temperatures of 8° and 12°. Half of the pots in each of these cases were removed to the warm greenhouse and kept there for eight hours each day (8 a. m. to 4 p. m.). These last two sets were used for the purpose of checking the results in experiment 5, Table 8. The results checked admirably with those shown in experiment 5, Table 8.

TABLE 9.—*Influence of various periods of alternating temperatures and their relation to light in the hardening process of alfalfas*

Length of time in hardening room each day [a]	Period of day in hardening room	Survival after freezing [b]		Length of time in hardening room each day [a]	Period of day in hardening room	Survival after freezing [b]	
		Turkestan	Arizona common			Turkestan	Arizona common
Hours		*Per cent*	*Per cent*	*Hours*		*Per cent*	*Per cent*
2	8 a. m. to 10 a. m.	77	66	16	4 p. m. to 8 a. m.	95	38
4	8 a. m. to 12 m.	90	43	24	Continuously	80	65
8	8 a. m. to 4 p. m.	75	55	8	8 a. m. to 4 p. m.[c]	41	15

[a] The hardening room was held at 3.8° C.
[b] After 2 weeks of treatment, all the plants were frozen 5½ hours at −13.9° C.
[c] This set was taken directly from the hardening room to a dark, warm case; the other sets were exposed to normal day length.

At the end of 14 days under the conditions described above the plants were frozen in the artificial freezing room. Those that had been in the warm greenhouse during the day had a healthy dark-green color, whereas those that had been in the hardening room during the day and in the dark chamber the rest of the time were light green. The plants continuously in the hardening room were not so dark a green as those that had been eight hours in the warm greenhouse during the day. The other sets were of a uniformly dark-green color.

With reference to the results from the Turkestan variety (Table 9), it is evident that the 2-hour exposure in the hardening room was not sufficient to give optimum hardening. Especially interesting is the fact that the plants kept in the hardening room 8 hours during the day did not have so high a survival as those kept 4 or even 2 hours. These results might be explained on the basis of the short period of light remaining to the plants in the warm greenhouse. Further evidence in support of this explanation is the fact that the plants kept for 8 hours during the day in the hardening room and for the rest of the time in a warm dark room had a very much lower survival; in

fact, the lowest survival of any of the groups. Representative pots from these sets are shown in Figure 7.

Arizona common did not respond to the hardening conditions as did Turkestan (fig. 6), and the results were not so clear-cut. For instance, the survival in Arizona common after four hours' exposure in the hardening chamber was not so high as after two hours. This is contrary to the results with Turkestan. However, Arizona common and Turkestan showed a similar response with respect to the influence of the dark chamber, the plants that were taken directly to the dark room from eight hours in the hardening room being the lowest in percentage survival.

Harvey (*6*) reports that alternate equal exposures during hardening of 12 hours at 0° C. and 12 hours at 10° or at 20° produce greater hardiness than continuous exposures at the average of these temperatures, i. e., 5° and 10°, respectively. The results obtained in alfalfa experiments indicate a greater response to alternate temperatures than was obtained in experiments with cabbage.

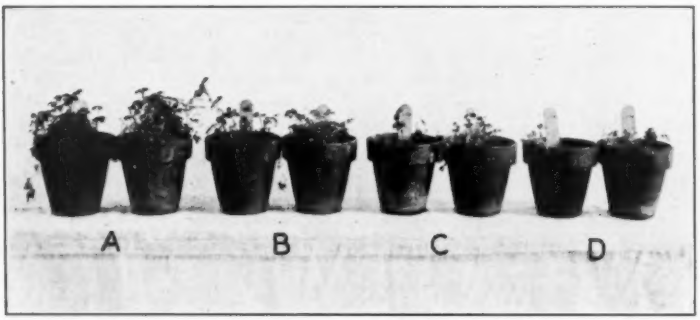

FIGURE 7.—Influence of alternating temperatures and of light on the ability of Turkestan alfalfa to resist cold: A, Two pots, 16 hours in the hardening room, 8 hours (8 a. m. to 4 p. m.) in warm greenhouse; B, 2 pots, continuously in hardening room; C, 2 pots, 8 hours (8 a. m. to 4 p. m.) in hardening room, rest of time in warm greenhouse; D, 2 pots, 8 hours (8 a. m. to 4 p. m.) in hardening room, rest of time in dark case in warm greenhouse. The average temperature of the hardening room during the 2-week period was 3.8° C. All pots were frozen for 5½ hours at −13.9°. Photographed two weeks after freezing

Table 10 gives the results of an experiment designed to show in greater detail the importance of the interrelations between light and temperature. All three sets of pots, each set containing eight pots of each variety, during a period of two weeks previous to freezing were exposed to seven hours of daylight, from 9 a. m. to 4 p. m., and for the rest of each day were kept in light-proof cases. The difference in treatment lies in the fact that each set was exposed to different conditions of temperature when exposed to light. One set when exposed to light was in the hardening room at 2° C., and the rest of the time in a dark warm case. This set showed the lack of photosynthetic activity by its yellowish-green color at the time of freezing and was decidedly the lowest in survival. A second set was kept continuously at 25°, but with only seven hours of light it was unable to resist the cold effectively, although it had a percentage of survival somewhat above that of the first set. This set was a lighter green than the third set, but not the yellowish green of the first. In contrast to these two sets, the third set, exposed to light at a temperature of 25° for seven

hours and placed in the hardening room at 2° for the rest of the time, had a very high percentage survival.

Thus alternating temperatures, with light during the warm periods, seem to furnish the optimum conditions for hardening.

TABLE 10.—*Influence of light and its relation to alternating temperatures in the hardening process of alfalfa*

Plants for 2 weeks previous to freezing kept in—	Survival after freezing [a]	
	Turkestan	Arizona common
	Per cent	*Per cent*
Light from 9 a. m. to 4 p. m. at 2° C.; dark case at 25° rest of time	5	2
Light from 9 a. m. to 4 p. m. at 25° C.; dark case at 25° rest of time	43	6
Light from 9 a. m. to 4 p. m. at 25° C.; dark case at 2° rest of time	91	77

[a] All plants were frozen 7 hours at −13.0° C.

INFLUENCE OF MOISTURE CONTENT OF THE SOIL ON THE HARDENING PROCESS

Rosa (*22*) reported that drought increased the resistance of cabbage to cold practically as much as low temperatures. Maximov (*16*) and Newton and Martin (*14*) suggested a close paralellism between drought and cold resistance.

An attempt was therefore made to determine what part drought plays in the hardening process. Plants grown in water-tight metal containers in the warm greenhouse at optimum soil moisture were hardened at different soil moistures. These metal containers were 4 inches square by 6 inches deep. Two hundred and fifty were filled with 1,000 g each of dry soil having a moisture equivalent of 23.8 per cent. The five varieties, Turkestan, Grimm, Nebraska common, Utah common, and Arizona common, were allowed to grow one month in the warm greenhouse under approximately optimum soil-moisture conditions. Just before the cans were taken to the hardening room they were allowed to dry until they contained about 14 per cent moisture. This process of drying out required three days. It was noticed that many plants showed incipient wilting at this moisture content.

When the soil was sufficiently dry, one-third of the metal containers were made up, by weighing, to a moisture content equal to three-fifths moisture equivalent, or 14.3 per cent; another one-third to 1 moisture equivalent, or 23.8 per cent; and the rest to 1⅔ moisture equivalent, or 33.3 per cent. All were then put in the hardening room, where they remained at an average temperature of 4.9° C. for 14 days. The moisture content of the soil was checked occasionally by weighing, and was kept relatively uniform, although it was impossible to keep it at the exact figure mentioned in the text.

At the end of the 2-week period the moisture in half of the metal containers at 14.3 per cent was made up to 33.3 per cent by adding the correct amount of water. Similarly, the moisture in half of those at 23.8 per cent was made up to 33.3 per cent. As soon as this operation was completed (in about 30 minutes) the cans were placed in the freezing chamber. By the time they were put in the freezer the water had percolated into the soil. All sets were frozen for the same length

of time, seven hours at −11.9° C. At the end of the 2-week period it was found that there was a great difference in the percentage survival of the different groups.

Since it is believed that there are no outstanding differences between the responses of the different varieties to this treatment, and since, by using the average of the varieties, a great many more individuals can be included, the discussion of results will be based on the average of all varieties given in the last column of Table 11.

TABLE 11.—*Influence of moisture content of the soil on the hardening process in alfalfas*

Experiment No. and period [a]	Moisture in soil		Freezing exposure			Replications in each variety	Survival						
	During hardening [b]	During freezing	Duration	Average temperature	Minimum soil temperature		Turkestan	Grimm	Nebraska common	Utah common	Arizona common	Ladak	Average
	P. ct.	*P. ct.*	*H. m.*	*°C.*	*°C.*	*No.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1, Nov. 23, 1929, to Jan. 29, 1930	14.3	14.3	7 0	−11.9	−11.2	7	75	52	20	57	37	.....	48
	14.3	33.3	7 0	−11.9	−3.8	6	87	72	65	97	80	.....	80
	23.8	23.8	7 0	−11.9	−5.4	7	71	86	72	76	79	.....	77
	23.8	33.3	7 0	−11.9	−3.8	6	95	82	89	85	82	.....	87
	33.3	33.3	7 0	−11.9	−3.8	16	76	66	70	85	73	.....	74
2, Dec. 10, 1929, to Feb. 20, 1930	15.0	15.0	4 30	−14.2	−9.4	9	.....	.....	53	47	.....	.....	50
	15.0	30.0	4 30	−14.2	−6.1	7	84	94	.....	.....	19	.....	66
	15.0	30.0	5 25	−14.4	−9.7	9	.....	.....	35	37	.....	.....	36
	30.0	30.0	4 30	−14.2	−6.1	8	95	74	78	65	19	.....	[c] 63 [d] 72
	30.0	30.0	5 25	−14.4	−9.7	9	.....	.....	25	41	.....	.....	33
3, Feb. 17 to Apr. 8, 1930	14.3	14.3	8 53	−15.6	−10.7	22	.....	19	.....	.....	.....	.....	.....
	14.3	35.0	8 53	−15.6	−3.8	22	.....	65	.....	.....	.....	.....	.....
	14.3	35.0	12 18	−16.5	.....	22	.....	39	.....	.....	.....	.....	.....
	23.8	23.8	8 53	−15.6	−6.4	22	.....	62	.....	.....	.....	.....	.....
	23.8	35.0	8 53	−15.6	−3.7	22	.....	72	.....	.....	.....	.....	.....
	23.8	35.0	12 18	−16.5	−9.9	22	.....	26	.....	.....	.....	.....	.....
	35.0	35.0	8 53	−15.6	−3.9	20	.....	86	.....	.....	.....	.....	.....
	35.0	35.0	12 18	−16.5	−15.5	20	.....	5	.....	.....	.....	.....	.....
4, Oct. 27 to Dec. 12, 1930	Dry.	[e] 13.0	3 0	−13.0	−8.2	5	8	15	.....	.....	36	67	32
	do.	[e] 45.0	3 0	−13.0	−4.0	5	92	86	.....	.....	88	89	89
	(*f*)	[e] 25.0	3 0	−13.0	−2.5	5	38	69	.....	.....	65	47	55
	(*f*)	[e] 45.0	3 0	−13.0	−2.5	5	94	97	.....	.....	84	71	87

[a] Date of seeding to date of freezing.
[b] In experiments 1, 2, and 3 the plants were hardened at a temperature of 1° to −5° C. during the 14-day period of special soil-moisture treatments. Experiment 4 was conducted at a room temperature of approximately 20°.
[c] Average of the varieties Turkestan, Grimm, and Arizona common.
[d] Average of the varieties Nebraska common and Utah common.
[e] Approximate percentage.
[f] Medium wet.

It may be seen from Table 11 that the plants in experiment 1, which were frozen at the low moisture content (14.3 per cent), had a much lower average percentage survival than any of the others. It may also be seen that the minimum soil temperature was much lower. No outstanding differences in percentage of survival are shown between the three sets frozen at 33.3 per cent moisture content, although they were hardened at greatly different moisture contents. In the set hardened at the high moisture content (33.3 per cent) the percentage of survival was slightly lower than in the others.

In order to determine whether the low percentage survival of the low-moisture group was due to the difference in soil temperature, experiment 2 was carried out in much the same way as experiment 1, except that at the time of freezing two sets instead of one were made

up to the high moisture content. Only two soil moistures (15 and 30 per cent) were used in this experiment.

When the freezing tests were made, one set of containers with low moisture and one set with high moisture were taken out at the same time; the other set with high moisture was allowed to remain in the freezer until the soil temperature approximated that of the low-moisture set.

Since different varieties were used in this test, a strict comparison can not be made between all groups, but a sufficient number may be made to show that those frozen at high moisture for the same period as those at low moisture were much higher in average percentage survival. On the other hand, among those frozen at the same moisture content there is a very slight difference in favor of those that were hardened at the low moisture content. However, no great significance can be attached to this difference.

Those that were frozen at a high moisture content for a longer period, until the soil temperature approached that of the low-moisture group, did not have quite so high an average percentage survival as those of the low-moisture group. The average percentage of survival among those that were frozen at the high moisture content was 36 and 33 per cent as compared with 50 per cent for those both hardened and frozen at a low moisture content. The soil temperature actually averaged slightly lower in the former group than in the latter.

In experiment 3 a somewhat larger number of cans was used, although only one variety, Grimm, was employed. As in experiment 2, two sets with high moisture were made up from the low-moisture group.

In experiment 3, there was a survival of 19, 62, and 86 per cent among the plants frozen for the same length of time with 14.3, 23.8, and 35 per cent moisture, respectively, and a survival of 65, 72, and 86 per cent for those from the low, medium, and high moisture groups, made up to high moisture content just before freezing and frozen for the same length of time as the previous group.

In the third group there was a survival of 39, 26, and 5 per cent with low, medium, and high moisture, respectively, when made up to high moisture and frozen for a long period of time. In this group there was a slight difference in favor of those hardened at the low soil-moisture content. The average of this group (23 per cent), representing the exposure calculated to give the same soil temperature as that with low moisture, corresponds closely to the 19 per cent survival for the low-moisture group. Representative plants from experiment 3, two weeks after freezing, are shown in Figure 8.

During the fall of 1930 a fourth experiment was undertaken. The plants were grown in pots and kept in a warm room at approximately 20° C. during the hardening period. A check was not kept on the percentage of moisture in the soil, but the pots of each variety were divided into two groups, one of which was watered normally and the other maintained as closely as possible at the incipient wilting point of the plants. This point could easily be determined by the slight wilting of the leaves which occurred in all plants of this group. At the end of two weeks of this treatment the plants were frozen, necessarily very gently, in the artificial freezing chamber. Immediately before freezing, half of the pots of each group were saturated with water,

but before the plants were completely frozen the excess water had had a chance to drain off.

The results in Table 11 show that decidedly greater killing occurred among the plants with low moisture content. The plants in the medium-wet soil survived considerably better, but not so well as those in the wet soil. There was very little difference between those hardened by drying and those watered normally, when frozen with the same moisture content; this difference was in favor of the low-moisture plants.

A very interesting point in connection with this experiment is that after the freezing there were some plants that had not been injured, the leaves remaining turgid. A careful tabulation of these showed 1 pot of Turkestan, 3 of Grimm, 3 of Arizona common, and 6 of Ladak. All of these were from the group that had been hardened by maintaining a low moisture content. However, even though the tops of some of the plants appeared uninjured, the average percentage survival for all the plants in this group was much lower than that of the

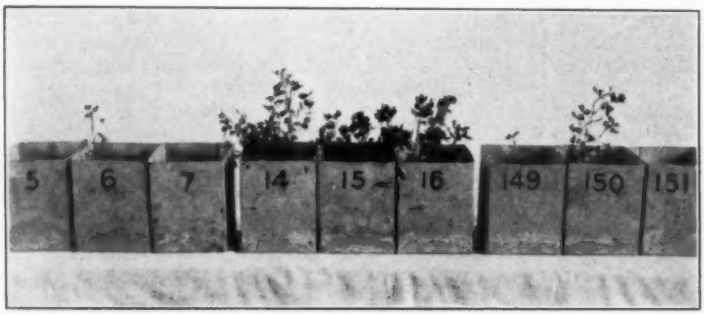

FIGURE 8.—Influence of hardening at different soil-moisture content on the ability of Grimm alfalfa to resist cold. Cans 5, 6, and 7, hardened at 14.3 per cent moisture content and frozen at the same moisture content for 8 hours and 25 minutes; cans 14, 15, and 16, hardened at 14.3 per cent moisture content and frozen at 35 per cent moisture content for 8 hours and 25 minutes. Cans 149, 150, and 151, hardened at 35 per cent moisture content and frozen at 35 per cent moisture content for 13 hours and 15 minutes. At this exposure the minimum soil temperature was the same as in cans 5, 6, and 7

other groups. In this experiment the freezing exposure was much less severe than in previous experiments, because there was no hardening at low temperatures, consequently this was the only experiment in which freezing was so slight that tops of plants remained uninjured. It is also true that the soil in the low-moisture group was somewhat drier in this experiment than in previous ones.

If the data from all four experiments are summarized certain results are outstanding. Most pronounced among them is the fact that plants growing in soil of low moisture content do not have nearly so good a chance of surviving a short period of cold as do those growing in soil having a higher moisture content. This seems to be due directly to the lower temperature in the low-moisture soil. Any hardening that may occur because of low soil moisture is entirely overshadowed by the difference in soil temperature.

In all four experiments, when the low moisture content was brought up to the high moisture content a slight difference in favor of the low moisture content was indicated in the percentage survival. This may indicate a slight degree of hardening at the low moisture content.

The evidence indicates that a low moisture content of the soil, even to the incipient wilting stage, does not serve to harden alfalfa to any great degree, not nearly enough to overcome the handicap of low-moisture content of the soil during freezing. These results are in agreement with those obtained by Klages (*10*).

The results no doubt serve to explain certain field observations which indicate that winter injury may be more severe during dry seasons. When it is remembered that actually, under normal conditions, the coldest weather is usually of short duration, it is quite possible that field results approach those obtained in the artificial freezing room.

INFLUENCE OF SEVERE WILTING

An experiment was made to determine the influence of severe wilting on the hardening process. In this experiment four flats of each

FIGURE 9.—Influence of severe wilting on the ability of alfalfa to resist cold. Kansas common alfalfa was sown in these flats January 30, 1931. Fourteen days before freezing, the flat on the left was allowed to dry, and the plants remained in a severely wilted condition until freezing. The flat on the right was watered normally. Both flats were frozen 48 hours at an average temperature of −6.5° C. Photographed three weeks after freezing

of three alfalfa varieties—Ladak, Kansas common, and Italian common—were planted January 30, 1931, and allowed to remain in the warm greenhouse. Fourteen days before freezing, half of the flats were allowed to dry. Although some water was added, the plants in these flats were kept in a severely wilted condition until they were frozen. The other flats were watered normally. The plants were frozen May 18, 1931, for 48 hours at −6.5° C. at the same moisture content that they had carried during the two weeks prior to freezing. This long freeze allowed the soil with normal moisture content to reach the air temperature and thus to reach the temperature of the soil with low-moisture content.

At the end of the 48-hour freezing period the estimated percentage survival of the wilted plants was as follows: Ladak, 85; Kansas common, 90; Italian common, 30. Of the plants that had been normally watered, all were completely killed. Figure 9 shows the difference

three weeks after freezing between two flats of Kansas common treated in the two different ways. The results of this experiment are in accord with those of Rosa (*22*) in his work with cabbage and of Martin (*11*) in his work with wheat.

RELATIVE IMPORTANCE OF THE INFLUENCE OF LIGHT, TEMPERATURE, AND SOIL MOISTURE ON THE HARDENING PROCESS

An analysis of the results in Table 1 shows that at a temperature of approximately 11° C. a change in length of day from about 10½ hours to 7 hours increased the average percentage survival of Turkestan 56 per cent, that of Grimm 22 per cent, and that of Arizona common 5 per cent. In other words, for each change of one hour in length of day at this temperature and within these limits, the percentage of survival increased by 16, 6.3, and 1.4 for Turkestan, Grimm, and Arizona common, respectively.

Table 6 shows that for a change of 1° C. at any temperature between 12° and 3° the average change in percentage survival is 12 for Turkestan, 10 for Grimm, and 6 for Arizona common.

With respect to moisture content of the soil, no such wide differences are apparent when the actual hardening of the plants is considered, except when the plants are severely wilted. Plants hardened for a period of two weeks near the incipient wilting point, as compared to check plants grown with normal moisture, showed so little increase in survival that no difference due to change in moisture content of the soil could be computed. On the other hand, there was a great difference in survival of plants frozen for relatively short periods at low or high soil-moisture content, as may be seen from Table 11. Arizona common, however, responded to as great a degree as Turkestan, and it is evident that the higher survival due to increased soil moisture during freezing is simply an effect of soil temperature.

The varieties used in the wilting experiment reported in Table 11 do not permit comparison with the light and temperature results; but it is clear that a slight variation in moisture content of the soil at or below the incipient wilting point of the plants exerts a marked influence on the resulting survival. This strong response is apparently not exerted at any soil-moisture content at which the leaves remain turgid.

In comparing the relative influence of light and temperature on the hardening process, it must be remembered that temperatures vary much more than length of day. However, considering the ranges of day length and of temperature that apparently affect the hardening process to the greatest extent, it is evident that both factors exert a very strong influence and that a change of one hour in length of day is very nearly equal to a change of 1° in temperature. Light coming as it does during the warmer portions of the day, appears to be of vital importance in maintaining the correct balance within the plant for optimum resistance. Moreover, it may be noted that varietal response to both light and temperature is very marked. A variety that responds most to change in length of day responds most to change in temperature, and, furthermore, this response is directly correlated with hardiness under field conditions in Nebraska, for the Turkestan variety, F. C. I. No. 15754, used in these tests is one of the most cold

resistant obtainable in that section (*19*). These data indicate the importance of hardening in bringing out greatest varietal differences in cold resistance.

DISCUSSION

The reason for the increased hardiness in the short-day plants is not clearly understood, but in this connection the work of Nightingale and others is suggestive. Nightingale (*15*) found that when plants were transferred from long-day to short-day conditions there was an enormous increase in soluble nitrogen in the short-day plants. Harvey (*5*) found an increase in the amino acid content of cabbage plants on hardening, and Janssen (*8*) showed some correlation between soluble nitrogen and winter hardiness. It should be remembered that in the present experiments the plants were under the short-day conditions only 14 days—insufficient time, perhaps, to reduce the carbohydrates greatly at the low temperatures prevailing. It is also possible that the lack of response to the short day at the high temperatures was due to the rapid utilization of sugars and starches, which would reduce

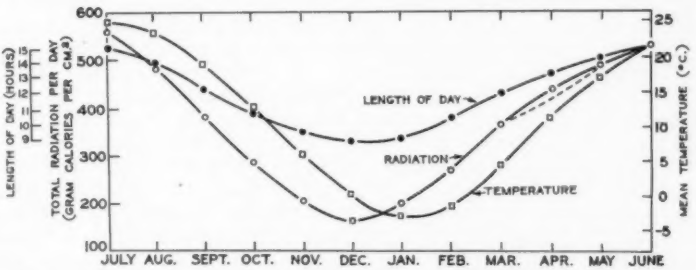
LENGTH OF DAY (HOURS)
TOTAL RADIATION PER DAY (GRAM CALORIES PER CM.²)
MEAN TEMPERATURE (°C.)
LENGTH OF DAY
RADIATION
TEMPERATURE
JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY JUNE

FIGURE 10.—Relation between length of day, average total solar radiation per day, and mean monthly temperature at Lincoln, Nebr., for a period of 12 years

the resistance of the plants to cold. A great decrease in these substances was found by Nightingale in the tomato when plants were transferred from a long day (14 hours) to a short day (7 hours). The low percentage survival of the short-day plants at 25° C. may indicate that photosynthesis was not able to balance the rapid respiration.

The results of these investigations emphasize the remarkable correlation between natural conditions and plant responses. In Figure 10 a striking similarity is observed between the curves for length of day and mean temperature. This figure also shows that temperature lags approximately one month behind length-of-day changes. While the total radiation naturally depends somewhat on the length of day, it also depends on the cloudiness and on the angle of incidence of the sun's rays. Thus, while the length of day varies from 15.1 hours as an average for June to 9.3 hours for December, the solar radiation varies from 566.9 gram calories per square centimeter per day as an average for July to 166.2 gram calories per square centimeter per day for December. If June is considered as 100 per cent in both length of day and solar radiation, December would be 61.6 and 30.4 per cent, respectively.

It may be noticed that solar radiation from the standpoint of time is more closely correlated with length of day than is temperature, and

that, from the standpoint of maximum variation, solar radiation is more closely correlated with temperature than is length of day.

The foregoing experiments show that the optimum response to the short day is at approximately 10° to 12° C. The days rapidly shorten in October and November, and during this period the average temperature is precisely at this range. (Fig. 10.) Another and perhaps important fact is that while temperatures may vary greatly from day to day and year to year the stimulating effect of the shortening day length on the hardening process is present, and in addition the shortest day lengths precede the lowest temperatures by approximately one month.

An additional similarity between natural environment and the controlled environment found to give optimum hardening is the alternating-temperature effect with light during the warmest portion of the day. It has been noted that under the conditions of this study the alternating temperature of 16 hours in the hardening room and 8 hours in the warm greenhouse during daylight developed greater hardiness in the plants than did continuous low temperatures. Similarly, under natural conditions, the light effect reaches a maximum early in the fall, at relatively high temperatures, and the alternating-temperature effect is greatest later in the fall and in early winter, at lower temperatures.

These studies show very clearly, at least with respect to the three varieties Turkestan, Grimm, and Arizona common, that hardening brings out greater varietal differentiation in resistance to cold.

Since alternating temperatures have been found to exert an important hardening effect, it may be desirable to use controlled alternating temperatures for hardening. Shortened day length for hardening may also be used to advantage under certain conditions. Both temperatures and light are of primary importance in producing optimum conditions for the hardening of alfalfas.

SUMMARY

This paper presents a study of the influence of light, temperature and soil moisture on the ability of different alfalfas to survive cold.

Alfalfa plants of the Turkestan, Grimm, and Arizona common varieties, grown for 30 days in the warm greenhouse, were used in the investigation, and a 14-day period was taken as the standard time in the hardening room.

Length of day was found to have a very important influence on the hardening process. The hardier varieties responded markedly to a short day length, under which they hardened off much more than under a normal day length. This response, however, was confined to certain temperature ranges. Practically no response to a 7-hour day at 20° or at 0° C. was observed, but at a temperature of 10° to 12° the response was very marked. Of the three varieties studied, the hardiest responded most to the short day; the unhardy responded least. In addition to length of day, intensity of light was found to be an important factor in the hardening process. This was important, however, only when the light reached a minimum beyond which a reduction caused a weakening of the plant. Above this minimum the response to light intensity was not so marked as the response to day length.

The short day at both medium and high temperatures greatly reduced growth. This reduced growth was reflected in a high percentage survival of plants at the medium temperatures, but not at the high temperatures, indicating that reduced growth is not a criterion of hardening.

Plants hardened under white light were better able to withstand cold than those hardened under red or blue light.

The resistance of plants to freezing varied with the temperature at which they were hardened. From 20° to about 0° C. the percentage survival increased as the temperature was lowered. Plants taken directly from a warm room to a hardening room at −5° were killed outright. The greatest response to temperature occurred at about 5°. The varieties again responded in order of hardiness, Turkestan showing the greatest increase in hardiness for each 1° change in temperature, and Arizona common showing the least.

At the range of optimum activity, light seemed to influence hardening almost as much as temperature, because for each hour of shortening in day length from a 10½-hour day to a 7-hour day there was an increase in survival of 16, 6.3, and 1.4 per cent for Turkestan, Grimm, and Arizona common, respectively, while for each 1° C. of decrease in the hardening temperature the increase in survival was 12, 10, and 6 per cent for the same three varieties. Hardening served to bring out greater varietal differentiation in resistance to cold.

Alternating temperatures during the hardening process markedly increased cold resistance, although this was influenced also by the time of day that plants were subjected to the cold and by the temperature of the hardening room.

Plants subjected to zero temperatures for 16 hours and then placed in a warm greenhouse (approximately 20° C.) during the day for eight hours developed much greater hardiness than those kept continuously at 0°. This was true at all temperatures up to approximately 5° for Arizona common and 7° for Turkestan. At these temperatures no increased benefits were obtained by alternating temperatures. At 12° a slight decrease in hardiness was found in plants subjected to a high temperature for eight hours during the day.

Two or four hours in a hardening room at 3.8° C. greatly increased the hardiness of the plants, the 4-hour plants being about equal in hardiness to those kept continuously at 3.8°; but these conditions did not produce as great a degree of hardiness as when the plants were kept in the hardening room for 16 hours and the remaining 8 hours in the warm greenhouse in daylight. Two or four hours did, however, give hardier plants than 8 hours in the hardening room, when this 8-hour period was taken during the middle of the day and the plants allowed to remain in the warm greenhouse during the night. The least hardy plants were those subjected to the low temperature for 8 hours during the day and for the rest of the time kept in a dark warm room.

Reducing the soil moisture as low as the incipient wilting point did not markedly harden any of the varieties. The rapidity and degree of freezing of the dry soil as compared to the moist soil far overshadowed any hardening effect of the low moisture. The plants in the soil containing the highest percentage of moisture invariably gave the highest percentage of survival when frozen for the same length of time as the low-moisture set. If they were frozen until the soil

temperature in the high-moisture set was the same as in the low-moisture set the survival was slightly in favor of the plants hardened in the low-moisture soil. Plants kept severely wilted for 10 to 14 days and then frozen in the severely wilted condition were very much more resistant to cold than those watered normally.

The close correlation between natural conditions in the fall, such as decreasing day length and alternating temperatures, and the effect on the hardening process of similar factors under controlled conditions is pointed out.

It is suggested that alternating temperatures or decreasing day length, or both, might be used under an artificially controlled environment for hardening plants that are to be frozen by artificially produced low temperatures.

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LARVAL INSTARS AND FEEDING OF THE BLACK CUTWORM, AGROTIS YPSILON ROTT.[1]

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INTRODUCTION

The studies of the black cutworm, *Agrotis ypsilon* Rott., here described, were undertaken because of outbreaks of the insect which occurred in the "overflow land" of the Black River in the neighborhood of Hoxie, Ark., following inundations that affected thousands of acres of cornland in 1927 and 1928. The experimental work was carried on at the field laboratory of the Bureau of Entomology in Webster Groves, Mo.

NUMBER OF GENERATIONS

The investigations indicate that there are three generations annually, the adults emerging early in April or May from pupae formed in the fall. The individuals in groups 28727 and 28730 developed from larvae collected in the field in the Hoxie district in 1928. Numerous adults were obtained in October of that year, and some lived until December, but no eggs were produced by them. Those in group 29033–b were from a female captured May 14, 1929. Those in groups 29111 and 29127 were from group 29033–b. Three definite broods were reared from this stock. None of the adults obtained in these experiments survived the winter.

METHODS

The cages in which the adults were held for oviposition consisted of inverted jelly glasses set in 4-ounce tin boxes, of 2-ounce tin boxes covered by jelly glasses, and of lantern globes with cheesecloth tops and saucer bases.

Eggs were collected daily in the early forenoon, and usually again in the late afternoon, and were kept under frequent observation in closed 2-ounce tin boxes. The newly hatched larvae were caged in 9 by 35 mm culture tubes having tight cotton or cork stoppers. Corn foliage was used for feeding experiments.[3] In the small culture tubes cross sections of corn leaves about one-quarter inch long were used. As the larvae grew and were less likely to be lost, they were placed in 25 by 100 mm shell vials, and when more than half grown were placed in 4-ounce tins. When it seemed apparent that the larvae were

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[2] The writer was assisted by R. C. Lange, assistant scientific aid, and, in the summer of 1929, by James B. Gahan and Dorothy Boyer, in the experimental work described in this paper.

[3] An exception occurred in the night of May 26, when the larvae in group 29033–b were hatching. Each set was given a piece of wheat leaf at 9.10 p. m., which was exchanged for corn foliage by 9.30 a. m. the next day. Micrometer caliper measurements revealed a thickness of 0.09 mm at the edge and 0.11 mm in the blade in both corn and wheat leaf blades, May 27. The quantities eaten were very small and were prorated for each set.

through feeding, sifted soil was added to the tins to permit the larvae to burrow in and form earthen cocoons for pupation.

The partly consumed foliage was removed from the cage once daily and pressed to keep it flat until it could be measured. Rarely, when none had been eaten, the foliage was left another day. In the later instars the supply had to be replenished more than once a day. Each piece of corn leaf blade was cut accurately to a prescribed length and was removed while its outline remained discernible. As the larvae increased in feeding capacity, it was necessary to record the number of pieces given, together with their lengths and widths, as an entire piece might be eaten and thus escape record.

Areas consumed were determined by the use of cross-section paper ruled to hundredths of a square inch. The original contours of pieces of foliage were determined by records and by their remaining edges. By these means the quantity consumed was quickly computed.

Head measurements were made preferably with micrometer calipers adjusted to read 0.01 mm. Measurements made with the aid of the eyepiece of the microscope were less dependable, apparently because of personal error in choosing the plane at which to make the measurement, but this method was used generally until after the second instar. While being measured with the calipers, the larva was held on a moistened camel's-hair brush, when possible, but older larvae were held between the finger and thumb. All measurements were made on the upturn, a procedure which eliminated the error due to the looseness of the caliper screw, as the instrument was set to begin on the upturn at zero.

DESCRIPTION OF SEVENTH AND EIGHTH LARVAL INSTARS

Crumb (*1, p. 55–56*)[4] published descriptions of the first to the sixth instars, apparently having encountered no records of 7-instar or 8-instar larvae. The following descriptions[5] were written from a study of one larva of each of the seventh and eighth instars in life, the specimens being immediately killed and preserved for reference. Ridgway's color key[6] was used for determining the colors recorded.

SEVENTH INSTAR.—Head 3.62 mm broad. Extended body 5.48 mm thick, 50 mm long. Cervical shield olivaceous black with narrow median line pale gull gray, and a pale spot at cephalic margin about one-sixth the width of shield from each side. Frons verona brown; major color on epicranium fuscous black on verona-brown background. Ocelli 1, 2, and 5 translucent, appearing whitish; 6 nearly so; 3 and 4 dark. Median line much interrupted, almost wanting on third thoracic and first abdominal segments, vetiver green, as also most of the limited light color of dorsum. Setal spots Ia and IIa blackish slate, each of the II series spots bordered mesad by spots of olive buff. Venter dark grayish olive. Ground color of anal plate dark olive buff, overlaid in part on each side of median line with deep olive. The encircling zone of setal spots III, IV, and V, and setal spot V, pale olive gray, setal spots III and IV olive gray.

EIGHTH INSTAR.—Head 3.84 mm broad. Body 5 mm thick, about 45 mm long. Neck full, as if nearing a molt. General color dark olive green with pale olive buff at mesal bases of series of II setal spots, with pale gull gray encircling bases of setae III, IV, and V, with V spot itself gleaming white. Head: Frons and region of eye and antenna verona brown; upper and middle epicranial halves and an area low on side brownish olive, the latter an island on the verona-brown area. Ocelli 1, 2, and 5 translucent, appearing whitish, 3, 4, and 6 nearly the color of the

[4] Reference is made by number (italic) to Literature Cited, p. 530.

[5] The numerals used in these descriptions of the seventh and eighth instars correspond to those used by Crumb in his description of the first six instars and refer to Figures 5 and 13 in the bulletin on tobacco cutworms (*1*) which figure the larval head shield and setal chart, respectively, for *Feltia* spp.

[6] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

clypeus. Mandibles with four major teeth, the upper somewhat bifid, the lower with a small tooth remote from termination of main tooth. Labrum with two semicircular lobes separated by a narrow rounded notch, paler than frons. The rough epicranium shows the rounded spots far back. Cervical shield olivaceous black with narrow median line hayti gray, integument of neck pale gull gray. The median line back of cervical shield is broken. The areas representing the greatly reduced subdorsal bands are vetiver green, including two broken slender lines on sides. The light area at mesad bases of series II setal spots, the encircling zone of setal spots III, IV, and V, and the V setal spot in its entirety, pale gull gray. Border of median line and dark color of back dark olive gray. I and II setal spots slate black. Setal spots III and IV olive gray. Venter deep slate olive. Anal shield olivaceous black on warm buff, the latter showing in median line and at margins.

The character of skin in the seventh and eighth instars agrees entirely with the description given by Crumb for the sixth instar, except that the "convex, rounded, distinctly isolated, very coarse shiny granules" occasionally range in size up to 0.1 mm each. The adfrontal sutures reach the occipital foramen. Setal tubercle I is about 0.6 mm in longitudinal axis of larva, 0.9 mm wide, and that of II, 0.9 by 1.7 mm.

Setal tubercle I is slightly more than half the diameter, both longitudinal and transverse, of setal tubercle II, each definitely transverse. Tubercle III is slightly larger than I but with less of its surface pigmented. Basal portion of leg claw without tooth. About 20 crochets on each proleg.

LARVAL DEVELOPMENT AND DETERMINATION OF INSTARS

The development of the larva is indicated in Table 1, in which the head measurements of many specimens are averaged, first for all, then for only those becoming adults, then separately by sexes for individuals which became adults after 6 instars, then separately by sexes for individuals which became adults after 7 instars, and lastly for the few females which became adults after 8 instars. Of those reaching the adult stage, 57.4 per cent passed through 6 instars, 38.9 per cent required 7, and 3.7 per cent required 8 instars.

TABLE 1.—*Average head measurements for the entire series and for the individuals of each sex of the black cutworm that matured in 6, 7, and 8 instars, respectively, Webster Groves, Mo.*

Instar	Entire series		Individuals which became adults		Individuals which became adults in 6 instars				Individuals which became adults in 7 instars				Individuals which became adults in 8 instars (no males)	
					Males		Females		Males		Females		Females	
	Males and females	Average head width	Males and females	Average head width	Individuals	Average head width	Individuals	Average head width	Individuals	Average head width	Individuals	Average head width	Individuals	Average head width
	Number	*Mm*	*Number*	*Mm*	*Number*	*Mm*	*Number*	*Mm*	*Number*	*Mm*	*Number*	*Mm*	*Number*	*Mm*
First	60	0.29	34	0.29	17	0.29	8	0.29	5	0.28	4	0.29		
Second	120	.44	70	.45	20	.47	12	.48	20	.42	14	.42	4	0.41
Third	142	.78	85	.79	25	.80	20	.83	24	.77	12	.78	4	.73
Fourth	134	1.38	92	1.39	29	1.59	21	1.53	23	1.99	15	1.20	4	1.04
Fifth	125	2.12	96	2.14	31	2.26	24	2.30	23	1.99	15	1.97	3	1.70
Sixth	113	3.05	97	3.07	30	3.36	24	3.44	24	2.72	16	2.72	3	2.44
Seventh	51	3.67	45	3.61					24	3.69	17	3.72	4	3.25
Eighth	4	3.81	4	3.81									4	3.81

In the analysis of the average sizes of the head capsule for the several instars, it may be noted that the average of the aggregate in the instar regardless of maturity, and the average of those individuals which became adults regardless of sex or number of instars, check up very closely. The averages for the adults which developed completely in six instars and for those which finished in seven and eight instars are fairly approximate in size, both in the first instar and in the last instar, but they do not check up very closely in the intermediate instars, where the growth of the larvae which underwent seven and eight instars, respectively, was retarded as compared with the growth of the 6-instar larvae. The number of individuals in the 7-instar and 8-instar classes may be too small for consistent record for growth in those classes. In the larger class of the 6-instar individuals the averages are more significant. There is not much difference between the head measurements of the males and females in the first three instars, but after that the heads of the males appear to be generally larger than those of the females.

A further analysis of the head widths is given in Table 2, where the maximum and minimum widths of the head capsules are also shown. For the first and second instars the records cover all the larvae under observation. The measurements given for the remaining instars are of those larvae only which finally became adults.

TABLE 2.—*Minimum, maximum, and average head width for the various larval instars of the black cutworm, Webster Groves, Mo.*

Instar	Individuals measured	Width of head capsule			Remarks
		Minimum	Maximum	Average	
	Number	*Mm*	*Mm*	*Mm*	
First	60	0.26	0.35	0.292	Entire series.
Second	120	.36	.52	.442	Do.
Third	90	[a] .61	[a] 1.11	.794	Only those yielding adults.
Fourth	92	.77	2.07	1.390	Do.
Fifth	96	1.09	2.80	2.140	Do.
Sixth	97	1.47	3.94	3.066	Do.
Seventh	45	3.14	3.95	3.610	Do.

[a] The molts represented by the measurements of 0.61 and 1.11 mm occurred in a period of less than 3 days, during which only 1 examination was made.

The head capsule is the least variable major portion of the larva, and the cast mask is the most durable portion of the exuvium. The head, therefore, is a good guide for determining the instar. While the larva is still active, the ocelli may be observed in the new head behind the ocelli of the mask which is about to be cast. Feeding is suspended, often the major part of a day, before molting. By measuring the head promptly after each molt, and again within two or three days after the last observed molt, the instars may be fairly accurately traced. The observation of the shifting ocelli sometimes aided in detecting a stage of development in the instar after which feeding could not be expected. Suspended feeding before a molt was very often used for fixing the dividing line between the feeding of one instar and that of the next. The actual ecdysis was rarely witnessed.

Measurements were generally made with the microscope eyepiece until after the second instar, because of the difficulty of handling

small larvae when making measurements by calipers. Later instars were measured by caliper.

DURATION OF INSTARS

The cages were examined at least once a day, when the food was changed and any exuviae removed and studied, and any details of changes in progress that had taken place since the last examination were noted. Usually the molts occurred between these examinations, and the length of the instar was computed as lasting from an unknown point between two observations to a similar point between two later examinations. For example, an egg hatched between 9 and 9.10 p. m., May 26, and the larva cast its first exuvia between 9.30 a. m., May 28, and 9.30 a. m., May 29; the first-instar period or stadium was, therefore, between 36.33 hours and 60.5 hours. The mean time in this example is 48.41 hours for the duration of the first instar.

Table 3 gives the average range of each instar and of the larval period, by hatching groups, regardless of whether or not the larva reached the adult stage. The first two figures of the group number denote the year. The first two hatching groups from eggs laid September 4 and 5, 1928, hatched between 8.20 and 9 a. m. and 9 a. m. and 4 p. m., respectively, September 10. The third hatching group from eggs of August 27, 1928, hatched between 4 p. m., August 29, and 10.30 a. m., August 30. The explanation for the marked differences in average periods of duration of the first instar is presented under the discussion of effect of temperature. The duration of the instars as recorded may therefore be regarded as fairly representative for the St. Louis district during May, July, August, and September. The first six instars lasted approximately 3, 3, 3, 4, 5, and 7 days, respectively.

TABLE 3.—*Record of the duration of instars of the black cutworm for groups hatching at different times, Webster Groves, Mo., 1928–29*

Group No.	Time eggs were laid	Total of group	First instar		Second instar		Third instar		Fourth instar	
			Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar
		Number	*Number*	*Hours*	*Number*	*Hours*	*Number*	*Hours*	*Number*	*Hours*
28727	9.30 a. m., Sept. 4	6	6	32 to 55	6	28 to 75	4	35 to 92	3	83 to 140
	8 a. m., Sept. 5	10	10	30 to 36	10	32 to 83	10	45 to 99	9	66 to 131
28730	4.10 p. m., Aug. 27	12	12	255 to 290	11	117 to 176	9	120 to 178	8	118 to 172
29033–b	2 p. m., May 22, to 3.05 p. m., May 23.	35	35	61 to 96	35	26 to 75	32	41 to 90	31	48 to 93
		4	4	42 to 67	4	19 to 69	3	39 to 88	3	47 to 90
		4	4	61 to 86	4	6 to 60	4	23 to 71	4	50 to 96
		2	2	60 to 86	2	[a]0 to 52	2	23 to 70	2	51 to 95
		48	48	53 to 89	48	15 to 66	47	33 to 88	42	58 to 112
29111	10.30 a. m., July 4, to 9.15 a. m., July 5.	37	37	45 to 74	37	32 to 84	35	21 to 83	27	21 to 82
29127	10.35 a. m., July 8, to 9.15 a. m., July 9.	11	11	47 to 104	11	16 to 66	9	27 to 73	8	23 to 66
		17	17	27 to 72	17	22 to 74	15	29 to 75	13	25 to 71

[a] These larvae were examined May 29, 9.45 a. m., May 30, 11 a. m., and May 31, 1.45 p. m. It is evident that the duration of the second instar was less than 52 hours and that it was more than 0 hour. There are several instances of successive molts on successive days.

TABLE 3.—*Record of the duration of instars of the black cutworm for groups hatching at different times, Webster Groves, Mo., 1928–29*—Continued

Group No.	Fifth instar		Sixth instar		Seventh instar		Eighth instar		Total larval period	
	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of stage
	Number	*Hours*	*Number*	*Hours*	*Number*	*Hours*	*Number*	*Hours*	*Number*	*Hours*
28727	3	120 to 172	2	112 to 175	0		0		1	626 to 672
	6	105 to 160	5	125 to 189	2	129 to 193	0		[b] 4	621 to 678
28730	6	119 to 181	5	142 to 206	0		0		1	1,008 to 1,051
29033–b	29	65 to 110	25	142 to 185	5	147 to 187	0		23	543 to 570
	2	57 to 103	2	110 to 156	1	172 to 212	0		1	1,069 to 1,103
	4	70 to 119	4	106 to 150	2	172 to 213	0		4	536 to 551
	2	70 to 119	2	96 to 147	2	172 to 212	0		2	520 to 543
	40	71 to 111	37	113 to 185	5	178 to 226	0		37	531 to 558
29111	25	36 to 84	25	88 to 137	17	151 to 196	0		25	494 to 522
29127	8	48 to 98	8	53 to 100	8	280 to 326	3	180 to 218	8	548 to 602
	11	39 to 85	10	49 to 96	9	157 to 202	1	222 to 262	9	400 to 548

[b] These are three 6-instar and one 7-instar individuals.

The mean average duration of larval instars is given in Table 4.

TABLE 4.—*The mean average duration in hours of larval instars of the black cutworm by cage groups for all records in each instar and for those larvae which became adults in the 6-instar, 7-instar, or 8-instar classes, respectively, Webster Groves, Mo.*

Cage group	Series	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Seventh instar	Eighth instar	Total larval period [a]
May	Total	73	44	62	78	89	140			
	6-instar	74	42	61	81	99	182			539
	7-instar	70	34	56	74	65	89	179		678
July 4	Total	59	58	57	49	60	113			
	6-instar	65	61	65	50	81	185			506
	7-instar	56	53	43	49	52	84	172		501
July 8	Total	60	45	51	46	67	74	193		
	7-instar	58	41	51	41	67	81	197		532
	8-instar	54	47	48	48	66	52	78	210	614
August	Total	272	146	89	145	150	171			
	6-instar	248	(b)	(b)	109	95	240			1,030
September	Total	38	55	68	105	139	154	161		
	6-instar	47	59	84	176	139	201			647
	7-instar	40	23	43	121	146	105	204		681

[a] Not necessarily the total of the mean averages of the individual periods.
[b] Not determined.

A few individuals made records quite at variance with the mean average duration. One larva of the 6-instar class, hatching July 4, required more than 118 hours in the first instar. One larva of the 6-instar class, hatching in May, in the third instar required less than 32 hours.

Less than 49 hours for the sixth instar was required in the case of a larva of the 7-instar class that hatched in May.

There was no eighth instar in the July 4 or August groups. In the September group one larva attained the eighth instar but was killed to represent that instar.

The entire larval period, that is, from the date of hatching to pupation, lasted less than 492 hours in the case of one 6-instar individual, and less than 563 hours in that of one of the 7-instar larvae that hatched in May; less than 457 hours in that of one of the 6-instar larvae and

less than 482 hours in that of one of the 7-instar class that hatched July 4; less than 474 hours in that of one of the 7-instar; and less than 598 hours in that of one of the 8-instar individuals that hatched July 8.

EFFECT OF TEMPERATURE

The study of time variations in the development of the immature stages in relation to variations of temperature is on the basis of the published records of the St. Louis station of the United States Weather Bureau. The floor of the thermometer shelter is 737 feet above mean sea level, 10 feet above the main roof of the Railway Exchange Building, and about 264 feet above the street level. The records of temperature taken by the Weather Bureau are used because the laboratory instrument was out of service for several weeks during the period of the experiments.

A cool period from August 30 to September 6, 1928, retarded the first instars of the August group, whereas warm weather following September 10 allowed faster growth, as is shown in Table 3, where the mean durations of the first instar in the August group and September 4 series are, respectively, 272 and 43 hours. The retarding influence of cool weather prevailing when the larvae are in the first instar may, as manifested in this example, be felt throughout the life of the larva. The mean averages for the entire larval periods of the August group and of the September 4 series are, respectively, 1,029 and 649 hours, and for the entire cycle from adult to adult are 1,344 and 1,129 hours. This record of contrasting life-cycle reactions to less than optimum temperature in the first larval instar may account for some outbreak phenomena, and is in keeping with the sensitivity recorded by Crumb (*1, p. 54*), which appears automatically to preclude the successful hibernation of any forms at Nashville, Tenn., except pupae developing during a short period in the fall.

Two larvae in the September 4 series were followed through the several instars in relation to daily mean temperatures. The mean duration of the first instar and the mean average temperature for the period were, for the first, 40.47 hours, 78° F.; for the second, 63.8 hours, 79.5°. The mean duration of the second instar and the mean average temperature were 46.25 hours, 82.5°, and 90.25 hours, 76.5°, respectively; third instar, 58.05 hours, 77°, and 86.13 hours, 68°, respectively; fourth instar, 170.08 hours, 65°, and 82.75 hours, 61°, respectively; fifth instar, 131.38 hours, 57°; and 131.47 hours, 57°, respectively; sixth instar, identical records, 201.47 hours, 66°. These two records show relatively speedy development with the warmer temperatures in second and third instars, and the reverse in the first and fourth instars; the larvae made identical records in the fifth and sixth instars with the same temperatures, the preceding irregularities having brought them to the beginning of the fifth instar at the same time. This nonadherence to the generally accepted rule of acceleration with warmth and retardation with cooler temperatures is not understood.

FEEDING EXPERIMENTS

The feeding records are fairly represented in groups in Table 5. The grouping of feeding records in this table is made according to cage group and hatching date of eggs. The first line of averages concerns all of the individuals in a particular instar regardless of whether they became adults or not. Thus, in the series of 35 hatching in one period

in the May group, only 22 reached the pupal stage in six instars and 3 in seven instars and 1 larva was preserved in its eighth instar. The average for the entire larval period of the group, therefore, could not be obtained because of the irregularities both in number of specimens and of instars and because of the division into the two classes of 6-instar and 7-instar maturing larvae. The average quantities of foliage eaten by all larvae of each of the seven instars, however, are given for the three series of groups.

TABLE 5.—*Average quantities of food eaten by larvae of the black cutworm during the various instars, indicated by square inches of leaf surface, Webster Groves, Mo.*

Group No.	Date eggs were laid	First instar		Second instar		Third instar	
		Individuals	Foliage eaten [a]	Individuals	Foliage eaten	Individuals	Foliage eaten
		Number	*Sq. in.*	*Number*	*Sq. in*	*Number*	*Sq. in*
29033-b	May 22-23	35	0.044	35	0.119	33	0.528
		4	.026	4	.079	3	.321
		4	.051	4	.086	4	.300
		2	.026	2	.101	2	.495
		54	.042	49	.111	47	.511
29111	July 4-5	5	.046	5	.112	5	.329
29127	July 8-9	5	.024	5	.128	4	.270
Total or average		109	.041	104	.112	98	.483
Average quantity of foliage eaten by 65 larvae that eventually became adults			.037		.114		.540

Group No.	Date eggs were laid	Fourth instar		Fifth instar		Sixth instar		Seventh instar	
		Individuals	Foliage eaten	Individuals	Foliage eaten	Individuals	Foliage eaten	Individuals	Foliage eaten
		Number	*Sq. in.*	*Number*	*Sq. in.*	*Number*	*Sq. in.*	*Number*	*Sq. in.*
29033-b	May 22-23	32	1.51	29	7.56	26	48.69	3	63.82
		3	1.33	2	4.70	2	31.78	1	61.96
		4	1.36	4	4.24	4	36.36	2	60.36
		2	1.40	2	2.95	2	29.18	1	62.42
		43	1.57	40	6.15	38	49.19	6	53.38
29111	July 4-5	3	.68	2	2.61	2	25.29	1	42.88
29127	July 8-9	2	.95	2	2.57	2	15.65	2	28.70
Total or average		89	1.49	81	6.27	76	45.85	16	53.57
Average quantity to foliage eaten by 65 larvae that eventually became adults			1.55		6.01		46.48		[b] 52.53

[a] Average per individual in all cases in the table.

[b] Quantity eaten by 14 larvae.

In Table 6 are recorded the average quantities of foliage eaten in the several instars by all larvae which became adults in six instars, with separate averages for individuals which became males and females, in the May and July 4 groups. Inasmuch as only one individual in each of a number of the groups became an adult, several of the records are those of individuals.

TABLE 6.—*Average quantities of foliage consumed by larvae of the black cutworm, May and July 4 groups, that reached maturity after only six instars, and averages by sexes, Webster Groves, Mo.*

Record No.	Individuals	Foliage eaten by larvae of the—						
		First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Total
Larvae of the May group:	*Number*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*
1	19	0.044	0.13	0.51	1.71	7.97	55.90	66.26
2	1	.032	.07	.47	1.52	7.10	54.66	63.85
3	2	.027	.10	.38	1.26	4.62	58.30	64.69
4	1	.027	.13	.49	1.43	4.48	48.80	55.36
5	27	.035	.12	.50	1.68	6.33	56.61	65.27
Larvae of the July 4 group:								
6	1	.070	.20	.66	1.19	3.03	39.61	44.76
Total or average	51	.039	.12	.50	1.66	6.79	55.89	65.00
Averaged by sexes:								
Male	29	.039	.12	.51	1.69	7.12	52.79	62.62
Female	22	.040	.12	.49	1.62	6.35	59.52	68.14

[a] The five sets of records for the May group in Table 3 are represented in the five records here given.

In Table 7 are recorded the averages for all larvae yielding adults in seven instars, and the separate averages for individuals yielding males and females, in the May, July 4, and July 8 groups. On an average, the female is decidedly the heavier feeder. The records of feeding in the earlier instars are not constantly heavier for the females, but in the last three instars they are markedly heavier. The duration of the instar, and hence the quantity of food eaten, is dependent largely upon the prevailing temperature.

TABLE 7.—*Average quantities of foliage consumed by larvae of the black cutworm, May, July 4, and July 8 groups, that reached maturity after seven instars, and averages by sexes, Webster Groves, Mo.*

Record No.	Individuals	Foliage consumed by larvae of the—							
		First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Seventh instar	Total
Larvae of the May group: [a]	*Number*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*
1	2	0.028	0.10	0.53	0.94	4.27	16.58	64.44	86.89
2	1	.035	.09	.24	.97	2.30	8.90	61.96	74.49
3	2	.074	.06	.22	1.46	3.85	14.42	60.36	80.44
4	1	.024	.07	.50	1.38	1.42	9.56	62.42	75.37
5	5	.028	.06	.41	1.29	3.38	14.67	52.23	72.07
Larva of the July 4 group:									
6	1	.022	.03	.10	.43	2.19	10.97	42.83	56.57
Larvae of the July 8 group:									
7	2	.015	.15	.37	.94	2.58	15.64	28.72	48.42
Total or average	14	.032	.08	.37	1.14	3.16	14.00	52.53	71.31
Average by sexes:									
Male	7	.038	.07	.31	1.14	2.63	11.36	51.93	67.48
Female	7	.027	.09	.43	1.14	3.69	16.65	53.12	75.15

[a] The 5 sets of records for the May group in Table 3 are represented in the 5 records here given.

The progression in quantity of feeding done in each instar in both the 6-instar and the 7-instar maturing classes is shown in Figures 1 and 2. The averages for the 6-instar class (Table 6) are represented by squares in Figure 1, the outside square representing the total area of foliage consumed. The correct measures of these squares are 0.197, 0.35, 0.71, 1.29, 2.6, 7.34, and 8.06 inches. The averages for the 7-instar class (Table 7) are represented by the squares in Figure 2. The correct measurements of the sides are 0.18, 0.287, 0.61, 1.07, 1.77, 3.74, 7.2, and 8.44 inches, the outside square representing the total quantity eaten.

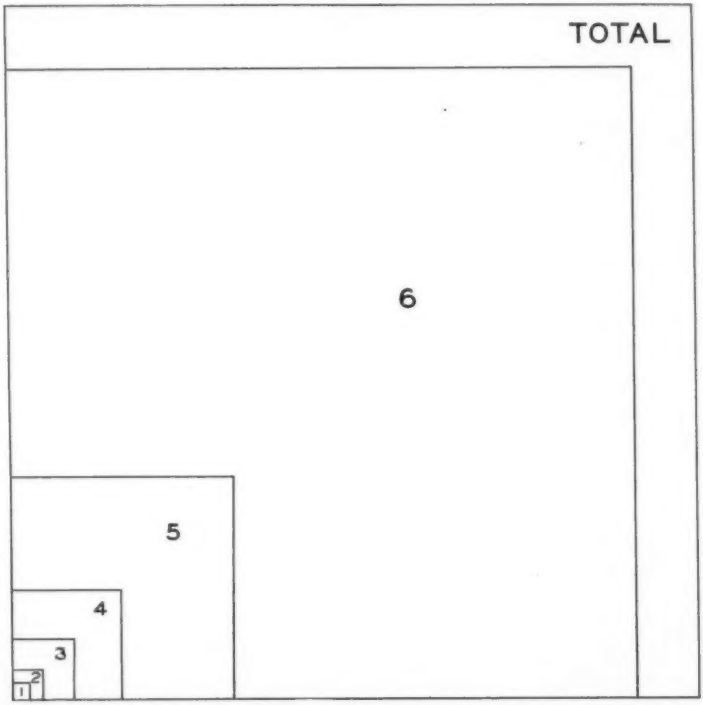

FIGURE 1.—Comparative area of foliage consumed in the successive instars by larvae of the black cutworm that matured in six instars. The numbers in the superimposed squares indicate the instars, and the outside square represents the total area consumed during the entire larval period

The entire larval period is approximately the same for the 6-instar and 7-instar larvae, but the quantity of food consumed is greater in the 7-instar than in the 6-instar class of the May group. The quantity of food consumed by the 7-instar larvae of the July 8 group is not a satisfactory record, as there were only two specimens in the class. However, the mean temperature for the period of the larval stages of the July 8 group, July 13 to August 3 (81° F.), was 8° higher than for that of the May group, May 23 to June 18 (73°). This would indicate that larvae go through the feeding stage with less aggregate feeding when the weather is warm than when it is cooler.

The progression in quantity of feed consumed and the time spent in each instar are shown graphically in Figure 3. The dotted line represents the May larvae maturing in 6 instars, the solid line the May larvae maturing in 7 instars, and the dash line the July 8 larvae maturing in 7 instars. Only the average duration of the instars and feeding records of the individuals which became adults were used.

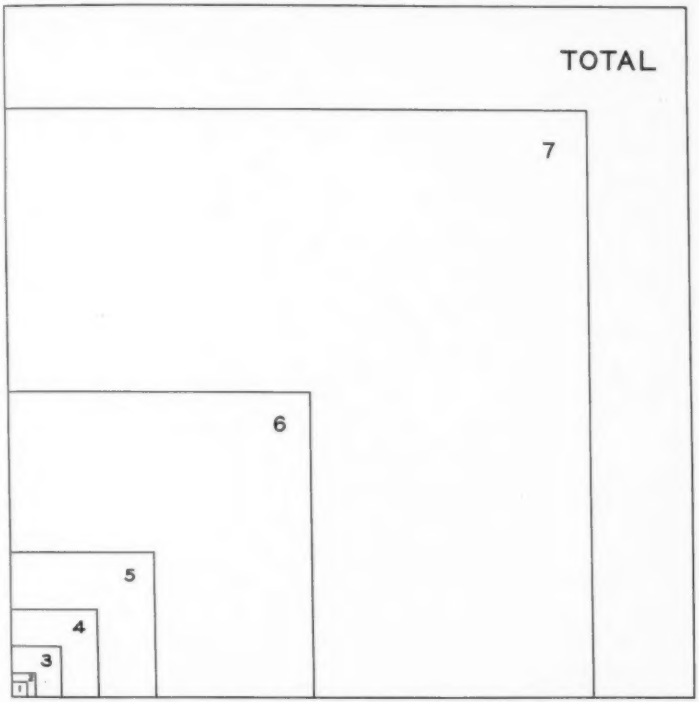

FIGURE 2.—Comparative area of foliage consumed in the successive instars by larvae of the black cutworm that matured in seven instars. The numbers in the superimposed squares indicate the instars, and the outside square represents the total area consumed during the entire larval period

DISCUSSION

Garman and Jewett (*4*) determined that the corn ear worm, *Heliothis obsoleta* Fab., had three complete broods a year at Lexington, Ky., the first brood (July) requiring 12 to 17 days to mature, the second brood (August) 12 to 19 days, and the third brood (September, 1908) 10 to 14 days and (September–October, 1909) 23 to 36 days. The instar periods averaged from 10 records of the first brood were, respectively, 2.63, 2.50, 2.08, 2.42, 2.58, and 3.58 days. The larval development was swiftest in warm weather and slowest in cool weather. The time of development of the last brood was normally lengthened by the cool weather.

Several writers on the instars of lepidopterous larvae of the cutworm family, or of larvae behaving much like the cutworms in their

method of work, including Davis and Satterthwait (*2*) on the true army worm, *Cirphis unipuncta* Haw., Parker, Strand, and Seamans (*7*) on the pale western cutworm, *Porosagrotis orthogonia* Morr., and Luginbill (*6*) on the fall army worm, *Laphygma frugiperda* S. and A., recorded the duration of the second, third, and fourth instars as shorter than that of the first, and that of the sixth as much longer.

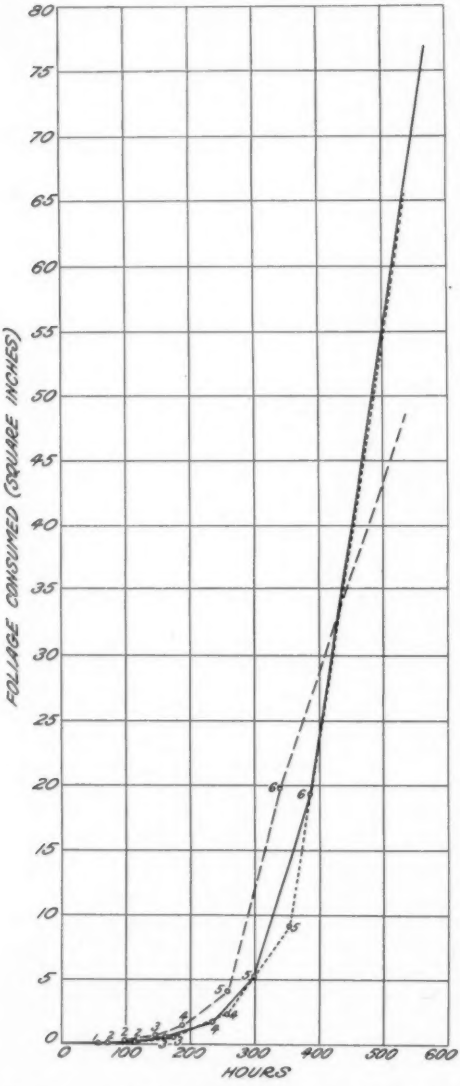

FIGURE 3.—Area of foliage consumed by the black cutworm in various larval instars. The dotted line represents the larvae of the May group maturing after 6 instars, the solid line those of the May group requiring 7 instars, and the broken line larvae of the July 8 group maturing in 7 instars. The figures at points where the lines change direction mark the close of the instars

Crumb (*1, p. 58*) states that in a series of tests on *Agrotis ypsilon* the six instars required, respectively, 2, 2, 3, 4, 4, and 5 days, a shorter duration for the first instar, in relation to that of the second, third, and fourth, than usual, but the usual substantially increased duration for the sixth.

In the present investigation of the black cutworm, effort was made to give all the larvae equally good food. Thus there is no recognized cause for the development of seven or eight instars in the several instances recorded. The record of Decker (*3, p. 144*) furnishes an entirely satisfactory explanation for the 7 to 11 instars in the 4-lined borer, *Luperina stipata* Morr., because of the continuous feeding of unfavorable parts of the plant to certain individuals. Crumb (*1, p. 134, 138*) found a seventh instar in the spotted-sided cutworm, *Agrotis badinodis* Grt., and the spotted cutworm, *Agrotis c-nigrum* L., but made no comment.

It is a common observation that a high temperature speeds feeding and growth and a low temperature checks feeding and growth. The present investigation included temperatures sufficiently low almost to suspend all development for a week at a time. Decker (*3, p. 146*) used controlled temperatures in certain experiments and reported that—

> Larvae reared in an incubator at 27° C. developed almost twice as fast as those reared at 20° C., about two and one-half times as fast as those reared in the screened laboratory, and about four times as fast as those reared at 13° C.

Where there are several generations a year, the duration of the last generation is likely to be lengthened by cooler weather. Garman and Jewett (*4, p. 539*) found three complete broods and a fractional fourth for the corn ear worm at Lexington, Ky. The three broods required approximately 30 days each. The fourth brood was definitely imperfect on account of frost.

Watson (*8, p. 1*) indicates that the late fall weather may affect the velvetbean caterpillar, *Anticarsia gemmatilis* Hbn., in the statement that "late in the fall, a few individuals molted seven times."

Parker, Strand, and Seamans (*7, p. 299*) indicate that the extreme variation in length of life of larvae of the pale western cutworm may be chargeable to food supply rather than low temperature, as the larvae migrate little or not at all, and may remain unharmed through lack of food for several weeks in the feeding season.

Luginbill (*6, p. 44*) records the fact that the larval period in the fall brood of the fall army worm at Columbia, S. C., averages 29 days, whereas that of the summer brood averages 14 days. This is obviously a slowing down with reduced temperatures.

Holloway, Haley, and Loftin (*5, p. 27–28*) furnish a tabulated summary showing the numbers of instars of the sugarcane borer, *Diatraea saccharalis* Fab., and numbers of examples in relation to average mean temperatures. Ten instars, the greatest number, was coincident with the lowest average mean temperature occurring in the experiment. Although seven instars occurred at an average mean temperature of 78.9° F., the prevalence of increased numbers of instars at average mean temperatures of 69.8° and lower marks a definite tendency to greater numbers of instars at the lower temperatures. There appeared also to be a slight tendency for instars to increase in number with the highest average mean temperatures. The length of the developmental period of the larvae was great, 242 or more days, at average mean temperatures of 61.4° and lower, and moderate to short, 19 to 76 days, at average mean temperatures of 65.3° and higher.

Crumb (*1*) shows the lengthening of the larval stage of *Agrotis ypsilon* (*p. 57*) at cooler temperatures in the fall and of the granulate cutworm, *Feltia annexa* Treit., (*p. 79–80*). In the variegated cutworm, *Lycophotia margaritosa saucia* Hbn. (*p. 112–113*), the reaction to cool weather was regular in the tardy larval development in the spring at average mean temperatures of 61° F. and lower, and in the summer, but the larval period lengthened beyond the spring periods at September average mean temperatures not lower than 73.5°.

Watson (*8*) measured the quantity of food consumed by weighing the foliage before and after feeding, preventing all appreciable evaporation by keeping leaf stems in water, and all under a bell jar.

Davis and Satterthwait (*2*) investigated the quantities of food consumed by larvae of the true army worm, using 153 individually caged larvae in September and October. The quantity of corn foliage

consumed in the several instars averaged, respectively, 0.03, 0.10, 0.34, 1.20, 5.36, and 34.13 square inches each, an average total of 41.39 square inches for each larva.

Luginbill (*6*) recorded the quantity of food consumed by larvae of the fall army worm. Two larvae were observed while feeding, corn leaves were used exclusively, and the quantity eaten was measured daily. One larva ate the equivalent of 90.2 cm^2 plus an area of 153 mm^2 skeletonized; the other ate 91.8 cm^2 of foliage and skeletonized an area of 192 mm^2. The fall army worm thus matures on about one-third the corn foliage required by the black cutworm and requires appreciably less time in the larval stage.

SUMMARY

There are three generations of the black cutworm a year at Webster Groves, Mo. Unseasonably cool weather may greatly retard larval development in the first instar and probably serves to increase the mortality rate in later stages of these larvae.

The growth of larvae in general, judged by head measurements, is approximate to one and a half times the size of the preceding head width, if the growth is completed in six instars. Where more instars occur, the increase after the sixth is greatly reduced for the subsequent stages. Increases in size between instars, especially in the first three, may be much greater; rarely the width in the second instar measures twice the width in the first.

The quantities of food eaten in the first three instars are small, then increase abruptly, especially between the fifth and sixth instars, in the individuals maturing in six instars. The measurement of food eaten was possible when corn foliage alone was supplied. In nature, the larvae bite off and then discard entire plants, thus getting an unknown volume of plant tissue and wasting perhaps a thousand times more than they consume. When fed corn foliage exclusively, a larva which undergoes six instars will eat the equivalent of 65 square inches of corn leaf.

The feeding of the larvae which eventually yield females is appreciably heavier than that of larvae which yield males, and is lighter in the July than in the May brood.

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DEVELOPMENT OF THE NODULAR WORM, OESOPHAGOSTOMUM LONGICAUDUM, IN THE PIG[1]

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PREVIOUS INVESTIGATIONS

Previous to 1925 nodular worms from domestic swine were considered as belonging to only one species, namely, *Oesophagostomum dentatum*, originally described by Rudolphi in 1803. In 1925 Goodey (*3*)[2] reported the occurrence in swine in New Guinea of a new species of this genus which he named *O. longicaudum*. In the same year Schwartz (*6*) found specimens of this form in collections of swine parasites from the United States, Tonkin (Indo-China), the Philippine Islands, the Fiji Islands, and China. These findings indicated a rather wide geographical distribution of the new species. In the course of investigations on swine parasites, Schwartz (*7*) noted that *O. longicaudum* is a common parasite of hogs in the southern part of the United States as far north as Maryland. In that State he found this species to occur rather infrequently, the majority of nodular worms which he collected from pigs raised in Maryland and in the vicinity of Washington, D. C., being *O. dentatum*. In contrast to the rather limited distribution of *O. longicaudum* in the United States, *O. dentatum* was found by Schwartz to be of frequent occurrence in hogs raised in the North as well as in those raised in the South. In addition to the difference in geographical distribution of the two species of Oesophagostomum in pigs, Schwartz (*7*) observed a wide difference in the type of intestinal lesion usually associated with the mature forms of the two species. Small uninflamed nodules, which were usually associated with mature forms of *O. dentatum*, were found in post-mortem examinations of pigs raised in Maryland and the vicinity of Washington, D. C. On the other hand, in post-mortem examinations of pigs raised near Moultrie, Ga., Schwartz observed not only small nodules similar to those found in the intestines of hogs raised in Maryland, but also large raised nodules each surrounded by an inflamed zone. In his discussion of the presence of the large inflamed nodules in southern hogs, Schwartz (*7, p. 413*) wrote as follows:

> The relatively large and conspicuous nodules noted in pigs killed at Moultrie, Georgia, have not been observed in the course of post-mortem examinations of pigs killed in the vicinity of Washington, D. C. Whether this can be accounted for on the basis of the occurrence of species of Oesophagostomum in pigs in the South, more particularly *O. longicaudum*, which are absent or rare in the vicinity of Washington, D. C., has not been definitely determined. Fourth-stage Oesophagostomum larvae obtained from intestinal nodules of pigs killed in Moultrie and other points in Georgia, have shown a morphology resembling that of the adult forms of *O. longicaudum*, and comparable larvae obtained from intestinal nodules of pigs slaughtered in the vicinity of Washington, D. C., have shown a

[1] Received for publication July 19, 1932; issued April, 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 542.

morphology suggestive of that of the adult forms of *O. dentatum*, particularly so far as the shape of the anterior end of the oesophagus and the relative length of the female tail were concerned.

In connection with these findings, the present writer made post-mortem examinations of 367 swine raised in Georgia and the surrounding States and observed that inflamed nodules were present in the intestines of animals infested with *Oesophagostomum longicaudum* alone, as well as in those having mixed infestations. Furthermore, *O. longicaudum* was the most common species of nodular worm found in the animals examined. This species was present in 98 per cent of the animals and *O. dentatum* in 81 per cent. These facts indicated a possible connection between the inflamed nodules commonly seen in southern hogs and the presence of *O. longicaudum* in the intestines of the infested animals, thus validating the suggestion made by Schwartz. In view of the opinion expressed by that writer regarding the economic importance of this pathological condition of the intestines, an attempt was made to obtain definite information on the development of *O. longicaudum* in the intestine of its host and to determine the pathogenesis of the lesion in question. To gain this information a series of infection experiments was undertaken.

EXPERIMENTAL PROCEDURE

The infection experiments described in this paper were carried out in Moultrie, Ga., in 1931. Seven pigs 6 to 8 weeks of age which had been raised free from nodular worms were infected experimentally with pure cultures of infective larvae of *Oesophagostomum longicaudum*. The host animals used had been farrowed and raised in a cement-floored pen which was cleaned and then scrubbed with boiling water several times each day throughout the experiments. The efficiency of this method in controlling extraneous infections with intestinal helminths is attested by the fact that no extraneous infections except such as were due to Strongyloides were found either in the control animals or in any of the experimental pigs involved in the tests.

Infective larvae of *Oesophagostomum longicaudum* were obtained by culturing ova from gravid females on moist sterile soil at room temperature for a period of five to seven days. The ova were obtained by cutting up the female worms fine with a pair of scissors. At the end of the culture period the infective larvae were recovered from the soil by means of the Baermann apparatus. In view of the fact that Goodey (*4*) had failed to bring about penetration of the skin by infective larvae of *O. dentatum*, the host animals in these experiments were infected by mouth, 500 to 700 larvae being given to each animal.

The pigs used were killed and examined 48 hours, 10, 17, 35, and 51 days after experimental infection.

INFECTION EXPERIMENTS

TEST 1

In order to determine the approximate time required by the larvae of *Oesophagostomum longicaudum* to reach the colon and penetrate the intestinal mucosa, the first post-mortem examination was made 48 hours after experimental infection. Numerous petechial hemorrhages were found in the mucosa of the caecum and large intestine.

Each petechial area, when examined under the microscope, appeared to be slightly elevated above the surrounding tissue, thus forming a nodule which contained a depressed area located centrally in the nodule. An early third-stage larva, inclosed in what appeared to be a connective-tissue cyst, was found in each lesion examined. Stained sections of these small nodules showed that the cysts, which were located either in the mucosa or submucosa, were surrounded by areas of intense inflammation with extensive liquefaction of the tissues, particularly between the cysts and the lumen of the intestine. (Fig. 1.) On close examination the cyst walls did not appear to be composed of connective tissue but of a rather tough, somewhat elastic, smooth, homogeneous substance, showing neither striations, nuclei, nor any other indication of cellular organization. In later stages of infection fibroblasts could easily be distinguished clinging to the outside of

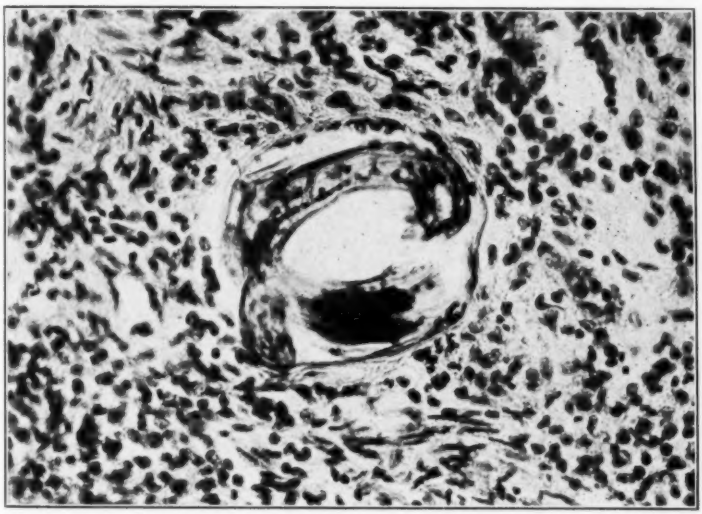

FIGURE 1.—Section through wall of large intestine, showing a cyst containing a larva of *Oesophagostomum longicaudum* 48 hours after experimental infection. × 600

the cyst walls. As is shown later, even in the case of empty cysts taken from an animal killed 17 days after infection, the cyst walls could easily be distinguished from the surrounding connective-tissue growth by the lack of cellular organization of the former.

The formation of the cysts may perhaps be due to a process similar to that described by Feng (*1*) in the case of cyst formations in the hedgehog, caused by *Physaloptera clausa*. Around the anterior end of this parasite, Feng observed a liquefaction of the host tissues, resulting in a homogenous zone where the nuclei failed to stain and the borders of the cells were not visible, a condition resembling somewhat the caseous zone caused by the action of tubercle bacilli. It may be that a somewhat similar process takes place in *Oesophagostomum longicaudum* infections. As the larvae penetrate the mucosa of the colon an extensive liquefaction of tissues occurs. The resulting

accumulation of serum, fibrin, and perhaps the remains of partly destroyed cells may undergo a process of coagulative necrosis resulting in a cystlike homogenous zone surrounding the parasite.

In addition to encysted larvae, a small number of third-stage Oesophagostomum larvae, which presumably had failed to penetrate the mucosa, were recovered from the lumen of the large intestine.

TEST 2

Ten days after infection of a second pig, post-mortem examination of the animal was made to determine the condition of the nodules and the degree of development which the parasites had attained during that period. Nodules raised approximately to a height of 2 mm above the surface of the mucosa and about 5 mm in diameter,

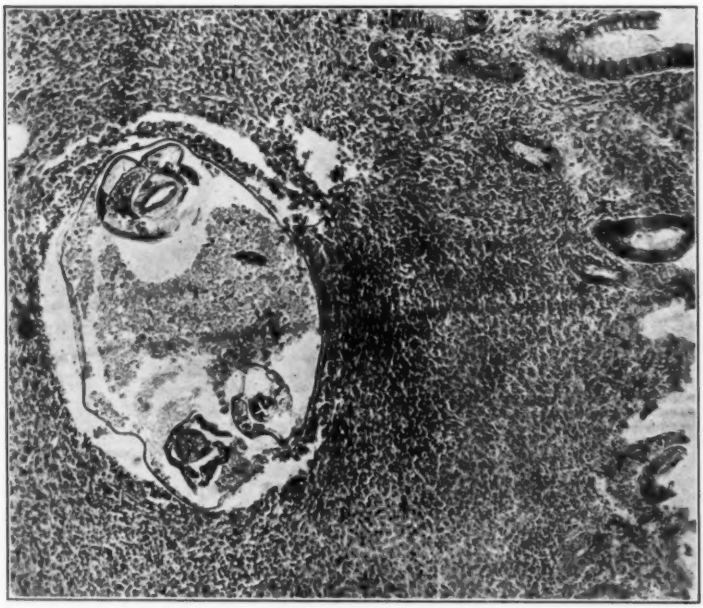

FIGURE 2.—Section through wall of large intestine, showing a cyst containing a larva of *Oesophagostomum longicaudum* 10 days after experimental infection. × 180

with a central depressed area and a surrounding inflammatory zone, were found in the caecum and large intestine of this animal. In press preparations, late third-stage larvae incased in cysts of a much larger size than those found in the earlier stage of infection could be seen within the nodules. The cysts, inside of which the parasites were freely movable, could be easily dissected out of the nodules. As determined in the course of dissection, the cyst walls were tough and somewhat elastic, with connective-tissue fibers clinging to the outer walls. In sections an extensive destruction of tissue could be seen to have taken place inside the nodules, resulting in what appeared to be a mass of fibrin, coagulated serum, and connective-tissue cells surrounding the cyst. (Fig. 2.)

As in test 1, no evidence could be found in test 2 which pointed to a connective-tissue origin of the cyst, the walls being easily distinguished from the surrounding fibroblasts. As can be seen in Figure 2, the cyst had ruptured in a number of places, perhaps because of pressure by the growing parasite, the gaps being filled, not by connective tissue, but by a substance resembling coagulated serum and fibrin. No appreciable change in thickness of the cyst wall was noted, the increase in size of the parasite perhaps being taken care of by the rupture and subsequent repair of the cyst wall.

No parasites were found in the lumen of the intestine of this animal.

TEST 3

On post-mortem examination of a pig 17 days after experimental infection, many fourth-stage larvae and early fifth-stage (mature) parasites were found in the lumen of the caecum and colon. Nodules from which the parasites had escaped were less inflamed, for the most part, and smaller than those that still contained larvae. A photomicrograph of a section through an empty nodule is shown in Figure 3.

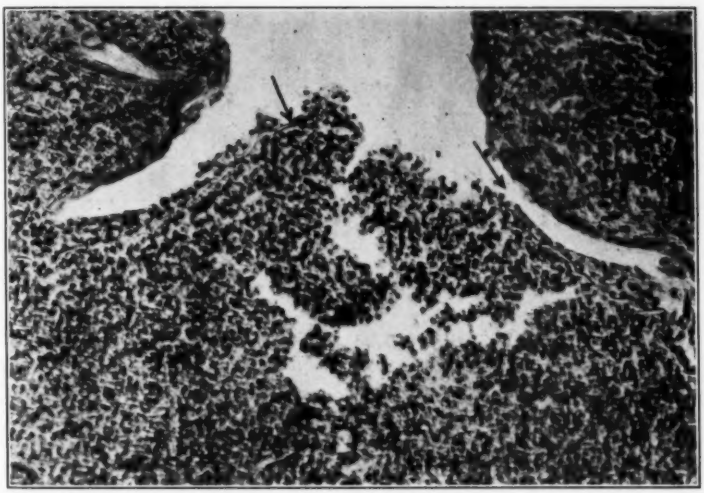

FIGURE 3.—Empty cyst produced by *Oesophagostomum longicaudum* 17 days after experimental infection. Arrows point to rupture in cyst wall. × 180

The cyst wall is broken, apparently having been ruptured when the parasite emerged from it to enter the lumen of the intestine. After the emergence of the larva the cyst apparently became filled with leucocytes, and the process of absorption of the cyst wall and its replacement by connective tissue, together with the regeneration of glandular tissue, was begun.

TEST 4

In a pig examined 35 days after infection, and approximately 18 days after the parasites had begun to enter the lumen of the intestine, the elevated and inflamed condition of the nodules had almost completely disappeared. In the animal examined post-mortem after this

interval, no raised nodules were found, the intestine being dotted with small areas of scar tissue which marked the location of the nodules in the earlier stages of the infection. Figure 4 shows a photomicrograph of a section through a nodule from a pig 35 days after experimental

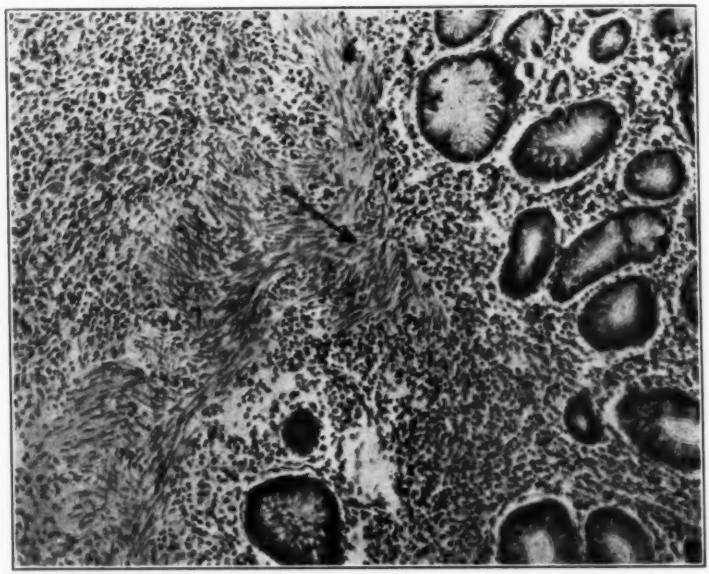

FIGURE 4.—Transverse section through a nodule in the colon of a pig 35 days after experimental infection with *Oesophagostomum longicaudum* showing the replacement of the cyst by connective tissue. Arrow shows area where cyst was located. × 180

infection. The cyst wall seems to have been completely absorbed, and only a mass of connective tissue, a few leucocytes, and an occasional giant cell remained to mark the location of the nodule.

TEST 5

Three pigs were kept to determine the length of time required for the development of *Oesophagostomum longicaudum* to fertile maturity. By means of the salt flotation technic and the charcoal culture method, the feces of each animal were studied every day, beginning the twenty-fifth day after experimental infection. In two of the animals, eggs were first noted in the feces 50 days after experimental infection. Charcoal cultures of the feces made at this time yielded infective Oesophagostomum larvae in seven days when the cultures were kept at room temperature in August. On post-mortem examination of the colon of one of these animals, numerous gravid females were found 51 days after infection. In the third animal, Oesophagostomum ova did not appear in the feces until the fifty-third day after experimental infection.

DESCRIPTION OF LARVAE

THIRD-STAGE LARVAE

Since the first and second stages of the larvae are preinfective, occurring outside the pig, the present investigation was begun with larvae in the third stage, which is the stage in which they enter the host. No parasitic larvae of this stage were recovered from any of the animals examined post-mortem during these experiments, with the exception of those noted in connection with test 1. These had not progressed, however, beyond the stage of free-living infective larvae. The free-living third-stage (infective) larvae were found to be similar in appearance and size to the corresponding stage of *Oesophagostomum dentatum* as described by Goodey (*2*). The infective larvae of *Oesophagostomum longicaudum* varied from 645 to 658μ in length and were approximately 30μ wide. These measurements include those of the sheath.

The infective larva lies free within the sheath, usually with the head pressed closely against the anterior end of this investment. The head resembles a truncated cone and has a terminal depression in which the mouth is located. The diameter of the esophagus is approximately the same throughout its length. The intestine, like that of *Oesophagostomum dentatum*, is composed of 16 cells, 8 on each side. These cells are filled with granular substances, the amount of which seems to decrease with the age of the larvae.

FOURTH-STAGE LARVAE

On post-mortem examination of a pig 17 days after experimental infection, fourth-stage larvae were found both free in the lumen of the intestine and in the act of emerging from the nodules. Considerable variation was observed not only in size and degree of development, but also in the proportion of length to width of these larvae. The smallest female measured was 2.7 mm long and 306μ in maximum width; the largest larva was 5.5 mm long and 288μ in maximum width. The males were as a rule smaller than the females, the smallest form being 1.9 mm long and 225μ in maximum width; the largest male was 4.2 mm long and 257μ in maximum width. Measurements of fourth-stage larvae are given in Table 1.

TABLE 1.—*Measurements* [a] *of fourth-stage larvae of Oesophagostomum longicaudum*

Item	Measurements of female No.—									
	1	2	3	4	5	6	7	8	9	10
Length of body	5.5	4.4	3.9	3.5	3.7	3.6	3.3	3.3	3.0	2.7
Length of esophagus	270	293	234	261	266	252	221	270	239	239
Length of buccal capsule	36	36	36	36	36	36	36	36	27	36
Distance from excretory pore to anterior end	158	162	144	135	149	149	176	144	135	135
Distance from vulva to anus	198	270	220	203	212	220	216	203	220	212
Length of tail	252	360	270	333	279	279	311	275	270	279
Distance from vulva to anterior end of reproductive system	477	887	297	368	90	198	522	293	103	190
Maximum body width	288	306	288	329	266	297	221	243	261	288
Maximum width of esophagus	68	68	68	54	63	72	68	90	50	90
Maximum width of buccal capsule	41	41	41	41	41	41	41	41	32	41
Body width at excretory pore	126	162	153	113	126	131	113	117	113	113
Body width at vulva	144	176	148	135	144	135	113	126	135	148
Body width at anus	68	81	72	77	77	72	86	72	72	72

[a] Measurements of body length are in millimeters; all other measurements are in microns.

TABLE 1.—*Measurements of fourth-stage larvae of Oesophagostomum longicaudum*—Continued

Item	Measurements of male No.—									
	11	12	13	14	15	16	17	18	19	20
Length of body	4.2	3.8	3.6	3.4	3.4	2.9	2.6	2.6	2.6	1.9
Length of esophagus	284	270	252	252	270	248	293	248	284	270
Length of buccal capsule	36	36	32	32	32	36	32	36	36	32
Distance from excretory pore to anterior end	203	149	149	158	131	99	117	144	140	122
Length of tail	149	153	158	153	167	140	158	135	122	167
Distance from anus to anterior end of reproductive system	1,340	1,200	1,370	1,300	1,100	1,179	1,013	1,022	810	900
Maximum body width	257	234	261	243	243	239	279	216	252	225
Maximum width of esophagus	68	72	68	68	63	54	68	63	54	68
Maximum width of buccal capsule	41	41	36	36	36	41	36	41	41	36
Body width at excretory pore	117	113	131	122	113	135	122	99	99	126
Body width at anus	90	90	99	90	104	99	81	81	59	77

As was observed by Goodey (*5*) for *Oesophagostomum dentatum*, there is a marked difference in the tails of the two sexes, the female tail being long and tapering gradually, whereas that of the male is short and tapers more abruptly. Both sexes are provided with a provisional buccal capsule which is slightly broader than long, the difference between the width and length being about 5μ in both sexes. (Table 1.) The walls are thick and heavily cuticularized. (Fig. 5.) Anteriorly each side of the buccal capsule narrows down to a point projecting inward. Posteriorly, after swelling out at first, each narrows to two conical points rather than to one point, as featured by Goodey for *Oesophagostomum dentatum*. These points are directed inward and apparently rest on a heavily cuticularized portion of the anterior end of the esophagus. A single, pointed tooth, apparently arising from the floor of the buccal cavity, projects into the cavity on the dorsal side. Morphologically the esophagus is similar to that of the adult, being more or less swollen at each end. The intestine and rectum are adult in appearance, and the three rectal glands can be seen in both sexes in the vicinity of the junction of the intestine and rectum.

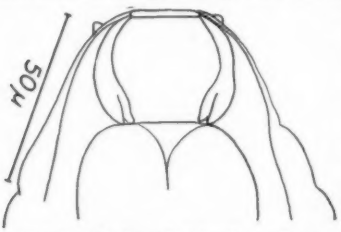

FIGURE 5.—Anterior end of fourth-stage larva of *Oesophagostomum longicaudum* showing shape of provisional buccal capsule.

A considerable variation in the degree of development of the genitalia of the female specimens was observed. The vagina appears to be formed from a mass of cells, probably ectodermal in origin, lying ventral to the ovejector apparatus, which is similar in shape to that of the adult worm. In the youngest forms studied, the vagina had not opened to the outside. In the larger, more developed forms the vulva had become visible, the opening extending through the cuticle of the worm. From the ovejector apparatus two rows of cells could be seen extending anteriorly to a distance of 90μ in the youngest specimen and 887μ in the oldest specimens. Figures 6 to 8 show some of the stages in the development of the female genitalia of this parasite.

The development of the reproductive system of the males of the fourth stage was rather difficult to follow. In all the specimens examined, a long band of cells could be seen lying dorsal to the rectum in much the same position as the spicules of the adult worm; these cells are probably the precursors of the spicules and gubernaculum. (Fig. 9.) The details of the formation of the male genital

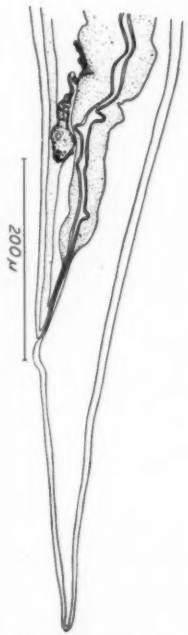

FIGURE 6.—Posterior end of female fourth-stage larva of *Oesophagostomum longicaudum* showing an early stage in the development of the posterior portion of the reproductive system

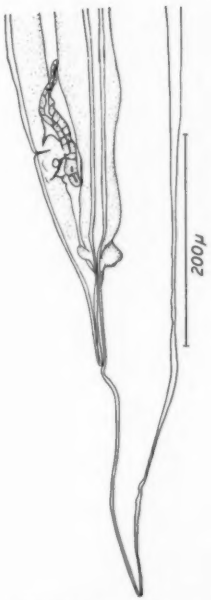

FIGURE 7.—Posterior end of female fourth-stage larva of *Oesophagostomum longicaudum* showing a later stage in the development of the posterior portion of the reproductive system

FIGURE 8.—Posterior end of female fourth-stage larva of *Oesophagostomum longicaudum* showing the posterior end of the reproductive system in a late stage of development

apparatus were not fully ascertained. As in the case of *Oesophagostomum dentatum*, a single band of cells could be seen on the ventral side stretching posteriorly from near the genital primordium to a point near the anus, where it turned and extended anteriorly again.

In the specimens studied, the developing bursa could be seen only in outline, its position being dorsolateral to the rectum. The bursal rays could not be clearly distinguished in any of the specimens of the fourth stage which were examined. However, two rows of clear spaces resembling vacuoles could be seen dorsolateral to the rectum;

the bursal rays were apparently formed from the tissue lying between these clear spaces. Figure 9, which is a camera lucida drawing of a developing bursa, gives as much detail as it was possible to make out in an examination of an entire specimen.

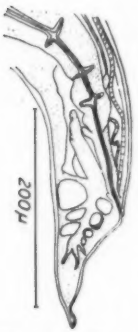

FIGURE 9.—Posterior end of early fourth-stage male larva of *Oesophagostomum longicaudum*

FIFTH-STAGE (ADULT) WORMS

Owing to the fact that only two larvae were found undergoing the fourth, or final, molt, the transition from the fourth to the fifth stages could not be definitely traced. The material of the definitive buccal capsule appears to be laid down in the space between the outer border of the provisional buccal capsule and the anterior end of the esophagus. The circumoral papillae appear to be formed in spaces near the middle of the provisional buccal capsule. The beginnings of the internal leaf crown, together with indications of the external leaf crown, can be seen on the anterior border of the developing definitive buccal capsule. Owing to the thickness of material and the large amount of débris through which it was necessary to focus, the exact nature and the relative positions of the adult characters were difficult to discern. Figure 10, which is a camera lucida drawing of a worm in an early stage of the fourth ecdysis, gives as much detail as it was possible to see. At the base of the buccal capsule, on each side, can be seen a mass of cuticularized material which is probably the developing definitive buccal capsule. On its anterior border the rudiments of the internal leaf crown can be distinguished.

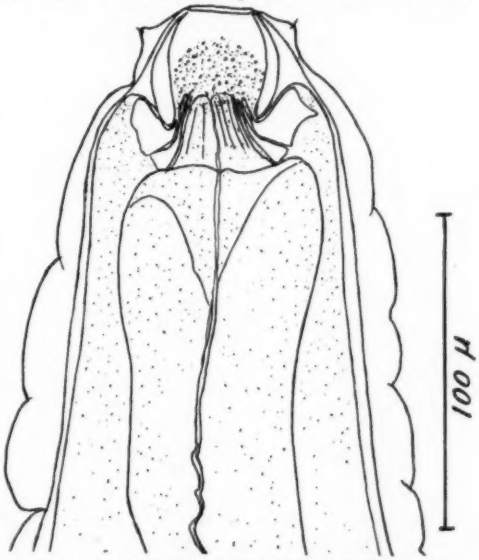

FIGURE 10.—Late fourth-stage larva of *Oesophagostomum longicaudum*, showing provisional buccal capsule together with the developing adult buccal capsule and the internal and external leaf crowns

In the late fourth stage of development the female reproductive organs have much the same form as in the adult stage, the principal change at molting apparently being that of size. (Tables 1 and 2.)

As no males were discovered in the process of molting, no description of changes in the male characters can be given.

TABLE 2.—*Measurements* [a] *of fifth-stage worms of Oesophagostomum longicaudum*

Item	Measurements of female No.—									
	1	2	3	4	5	6	7	8	9	10
Length of body	7.8	6.8	5.6	4.5	3.7	3.3	4.1	5.1	2.8	2.5
Length of esophagus	391	396	423	432	387	396	404	383	400	378
Length of buccal capsule	22	17	17	17	22	17	22	17	17	17
Distance from excretory pore to anterior end	168	142	180	180	189	142	142	215	185	180
Distance from vulva to anus	357	267	396	258	361	245	387	245	387	387
Length of vagina	146	135	135	176	116	135	135	73	135	73
Length of ovejector apparatus	219	151	206	237	151	151	198	151	241	151
Length of tail	487	482	351	360	243	318	353	312	430	306
Maximum body width	366	280	288	270	252	280	344	267	318	252
Maximum width of esophagus	139	112	108	126	108	90	120	90	112	90
Maximum width of buccal capsule	69	65	65	65	65	65	65	69	65	65
Body width at excretory pore	185	163	162	144	153	155	172	135	176	153
Body width at vulva	224	219	172	144	135	144	172	112	163	198
Body width at anus	112	116	90	99	90	56	73	73	86	63
Distance from vulva to anterior end of reproductive system	1,022	1,853	1,853	2,788	2,210	1,853	2,210	267	787	787

Item	Measurements of male No.—									
	11	12	13	14	15	16	17	18	19	20
Length of body	7.7	6.9	6.5	5.8	5.5	5.4	4.9	4.7	6.3	4.3
Length of esophagus	373	378	377	377	361	391	353	370	374	374
Length of buccal capsule	22	13	22	17	17	17	22	22	17	22
Distance from excretory pore to anterior end	164	181	181	168	181	176	146	133	176	163
Maximum body width	308	292	361	280	318	249	206	228	391	224
Maximum width of esophagus	103	108	108	103	120	99	99	99	113	103
Maximum width of buccal capsule	69	65	73	60	65	65	65	69	65	69
Body width at excretory pore	148	163	172	155	172	138	155	133	168	142
Body width at anus	127	146	155	181	155	125	133	116	155	99
Distance from anus to anterior end of reproductive system	4,318	4,420	3,859	3,366	2,727	3,332	2,890	2,601	4,420	1,904
Length of gubernaculum	107	99	108	90	95	86	103	108	90	86
Length of spicules	787	851	817	817	817	817	830	839	839	774

[a] Measurements of body length are in millimeters; all other measurements are in microns.

SUMMARY

Experiments designed to trace the development of the swine nodular worm, *Oesophagostomum longicaudum*, in its definitive host, were carried out by feeding pure cultures of infective larvae of this parasite to pigs raised free from nodular worms.

Forty-eight hours after experimental infection, encysted third-stage larvae were found in the colon of the infected animal; the cysts were surrounded by areas of intense inflammation with liquefaction of tissues.

In sections the cysts, the exact nature of which has not been definitely determined, appeared to be composed of a smooth homogeneous substance containing neither striations nor nuclei, and easily distinguishable from the surrounding tissue.

Inflamed nodules were found in the colon of an animal which had been infected 10 days prior to post-mortem examination. Within the nodules the parasites were inclosed in cysts which were much larger than those found in the earlier stages of infection. The cyst walls appeared to be of the same composition as those observed in the earlier stages of infection and were easily distinguishable from the surrounding fibroblasts. No decrease in thickness of the cyst walls

was noted, the increase in diameter of the cysts apparently being the result of rupture by the parasites, followed by subsequent repair.

On post-mortem examination of an animal 17 days after infection, fourth-stage larvae and early fifth-stage worms were recovered from the lumen of the caecum and large intestine. Late fourth-stage larvae were found in the nodules and in the act of emerging from the nodules, a fact which indicated that the larvae enter the lumen of the colon in the late fourth or early fifth stage of development. Nodules from which the parasites had emerged were smaller and less inflamed than those that still contained larvae. In sections, the cysts inside the empty nodules were seen to be broken, apparently having been ruptured by the parasite when it emerged.

After the emergence of the parasites, the nodules which at first were highly inflamed and contained a central necrotic area, appeared to decrease in size and in degree of inflammation. At the end of 35 days the nodules had almost completely disappeared, their location being marked, in section, by masses of connective tissue, leucocytes, and giant cells.

Sexual maturity, as indicated by the appearance of eggs in the feces of experimentally infected animals, was reached by the parasites in 50 to 53 days after infection.

A brief description is given of the larval stages recovered from the intestines of infected animals.

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SOME SUMMER-WOOD PERCENTAGE RELATIONSHIPS IN THE SOUTHERN PINES[1]

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INTRODUCTION

Summer wood, or that portion of the annual ring of wood formed in the latter part of the growing season, has a larger proportion of cell-wall substance per unit of volume than does the remainder, or spring-wood portion, of the ring. This imparts density to the timber and influences its uses. Many of the properties of wood can be correlated with its density as expressed by its specific gravity. Newlin and Wilson (*12*)[3] have presented such mathematical correlations in great detail. Additional correlations might include qualities of value in the production of wood pulp, such as yield, cellulose content, power and chemical consumption, and paper strength. The fact that the proportion of summer wood varies greatly has long been recognized, but the cause and effect and extent of these variations have had little attention from investigators. Yet this information is important for the most effective growth and utilization of timber.

In most of the investigations to date little has been attempted beyond the establishment of a correlation between width of summer wood, specific gravity, and rate of growth. Hartig (*6*) apparently was the first to show that the specific gravity of wood is influenced by the proportion of summer wood. Janka (*7*), working with larch, reached the same conclusion, but he claimed further that there was no relationship between rate of growth and specific gravity. Hale and Fensom (*5, p. 18*) state that "there is a tendency for white spruce with narrow rings to be heavier than wood with wide rings * * *." Klem (*9*) likewise points out that ring width is not correlated with specific gravity, especially if the wood comes from different regions with dissimilar growing conditions. He maintains, however, that the specific gravity of the wood increases as the percentage of summer wood in the ring becomes greater. Cieslar (*3*) noted that in spruce the higher specific gravity was found in wood which contained the greater ratio of summer wood to spring wood. Certain other of his conclusions, as presented later in this paper, indicate that he recognized a relation between rate of growth and density of wood. Paul (*13, p. 12*) went somewhat farther than the others in his conclusion:

> Both very wide and very narrow rings in conifers usually contain a large proportion of the spring-wood layer, so that in these species wood representing either extreme of growth may be low in specific gravity. The wood of intermediate growth is usually the heavier.

[1] Received for publication June 6, 1932; issued April, 1933.

[2] The author is indebted to B. H. Paul, of the U. S. Forest Products Laboratory, for the material which made this investigation possible, and to Arthur Koehler, of the same laboratory, for his helpful criticisms of the paper. The first of the curves was fitted by the class in advanced mathematics at Virginia Polytechnic Institute in the spring of 1930. Dean J. E. Williams was especially helpful in discussing and interpreting the curves, as well as in offering suggestions during the writing of this paper.

[3] Reference is made by number (italic) to Literature Cited, p. 516.

That the specific gravity of the wood of any one species is not necessarily dependent upon the percentage of summer wood is well known. The nature of the summer wood must be taken into consideration, as is pointed out by Koehler (*10, p. 131*), who emphasizes the fact that "the summer wood itself may vary considerably in density." The radial diameter of the cells (tracheids) in conifers and the thickness of the walls are the main factors influencing the density of this portion of the ring. Adams (*1*) notes that "the width of the annual ring is dependent upon size as well as number of tracheids." As tracheids increase in radial diameter, the thickness of their walls decreases, leading to a greater lowering of the density than would be anticipated from the increase in tracheid size alone. In addition, the density of the cell wall itself may vary to an appreciable degree, depending upon the amount of infiltrated materials. All of these factors militate against an unvarying correlation between specific gravity and the percentage of summer wood in the ring, but this percentage appears to be the simplest method of determining relative differences in the specific gravity of some species of wood. It is deemed a sufficiently reliable index of density to justify the methods used in this investigation.

Many investigators have shown, as stated by Paul (*13, p. 1*), that—

> the specific gravity * * * may be modified by controlling local factors which affect the growth either of forest stands or of individual forest trees, so that it becomes possible, within natural limits, to regulate the specific gravity of wood according to the particular use in view.

Paul has shown that trees on burned areas and those on sandy sites consistently produced wood of low specific gravity. The density of stocking was likewise a factor in the weight of wood, since open-grown trees produced lighter wood than crowded ones. Other investigators have found similar effects resulting from density of stocking. Cieslar (*3*) stated that rapidly growing dominant spruces produced wood of lower specific gravity than did codominant trees in the same stand. But the suppressed or crowded trees were not so consistent. In one instance the wood was higher in density and in another was comparable in specific gravity to that in the dominant trees. Bray and Paul (*2*) reported that the specific gravity of longleaf pine wood from trees of slow growth averaged 8 per cent higher than that of similar portions of rapid growth, and that, in the same stand, the unit weight of wood increased as the rate of growth decreased because of density of stocking.

That the distance aboveground exerts an influence on specific gravity is likewise evident. Bray and Paul (*2*) found that the wood of top logs of a medium rate of growth had a lower specific gravity than did any of the butt wood, regardless of its rate of growth. Hale and Fensom (*5*), on the other hand, noted an increasing density of white spruce wood with increased height aboveground, especially in levels above 30 feet. They suggest, however, that this may be specific for spruce in view of the results obtained by Sterns (*15*). This author noted a steady decrease in specific gravity in Douglas fir from the butt to the top of the tree.

Paul and Marts (*14*) concluded, as a result of three years' experiments with the effect of irrigation and commercial fertilizers on longleaf pine, that there was a fairly close correlation between current water supply and the formation of summer wood, that without irri-

gation a complete fertilizer increased the rate of diameter growth because of increases in the spring-wood zone, and a nitrate fertilizer produced diameter increases which were more evident in the summer wood than in the spring wood. Thus it seems to be established that the specific gravity of conifer woods is definitely affected by various factors, among them being position in the tree, density of the stand, and local site factors, such as soil and soil moisture. The present investigation was designed to show in more detail how the variation in the percentage of summer wood, which is responsible for the greatest variations in specific gravity, is brought about.

SOURCE OF MATERIAL

The longleaf pine (*Pinus palustris* Mill.) material upon which the greater part of this investigation is based was furnished by the United States Forest Products Laboratory. It had previously been used in another investigation by Bray and Paul (*2, p. 163*), and the following description is taken from their paper:

> The stand selected for study is located near Walnut Hill, Escambia County, Fla., in a former clearing which, according to local residents, was used for farm crops until 35 or 40 years ago. The age of the trees, as shown by the number of annual growth rings on the stumps, ranged from 30 to 35 years. The number of trees per acre varied from 300 or less in some places to about 1,300 in others. The material for this study was obtained from different portions of an area not exceeding 10 acres in extent. This area, comprising the summit of a low, rolling hill, was fairly level. The conditions of topography, soil, and other visible growth factors, except the number of trees per acre, were similar throughout the plots from which the pulpwood samples were cut.
>
> Representative plots from which the wood samples were to be cut were staked out in areas containing approximately 350, 600, and 1,200 trees per acre, respectively.

The trees in the plot containing 350 trees per acre ranged in breast-high diameter from 4 to 14 inches with an average of 8 inches. The trees from the plot with 600 trees per acre ranged from 3 to 10 inches in diameter with an average of 6.4 inches. Trees from the densely stocked plot ranged from 2 to 9 inches with an average diameter of 4.4 inches.

The loblolly pine (*Pinus taeda* L.) material was also furnished by the forest products laboratory. One group (No. 1326) came from Windsor, N. C., and another (No. 1324) from Franklin, Va. These were removed during logging and milling studies, and the density of stocking was not recorded. Since the trees in each group were about the same age, differences in diameter would be indicative of the amount of growing space available. The loblolly pine from each locality was accordingly sorted into two groups. The trees of small diameter were considered as representative of close spacing in the stand, and, conversely, those of large diameter were considered as representative of a more open stand.

PROCEDURE

The rings on each section were measured to the nearest hundredth millimeter along a radius from the outside toward the center. A measuring device designed and constructed in the Division of Forest Insects of the United States Bureau of Entomology was used, and the measurements were made continuously from the outside toward the center. In this way any error in measurement or computation could be detected. All curves were fitted by the method of least

squares. When two curves might appear to fit the experimental points the one selected was that in which the algebraic sum of the squares of the residuals most nearly approached zero. In no instance were the curves adjusted in any way.

VARIATIONS IN THE TWO PORTIONS OF THE ANNUAL RING

The percentage of summerwood in any one growth ring is the resultant of variations in either or both the spring wood and the summer wood. Thus the variations in the proportion of summer wood dependent upon stocking, position in the tree, and other factors can be interpreted only in relation to the corresponding variations in the width of the ring as a whole.

RELATION BETWEEN SUMMER-WOOD WIDTH AND RING WIDTH

No references to the relationship between either summer-wood or spring-wood width and total ring width have been found, except the

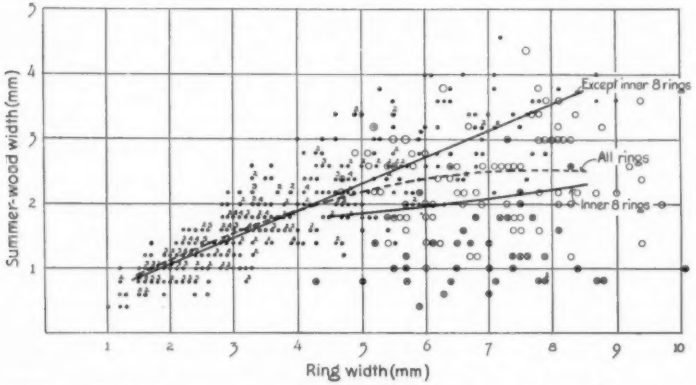

FIGURE 1.—Relationship between summer-wood width and ring width in longleaf pine at breast height in a stand with 350 trees per acre. Points for the eight inner rings represented by circles, for rings outside of the eighth by dots, and for the four innermost rings by crosses within circles. The small numerals indicate identical points

following statement by Forsaith (*4, p. 88*): "In coniferous species the summer wood is constant in respect to width and such variations as do occur are largely confined to the spring-wood portion of the ring"; and the statement by Paul (*13, p. 16*) which has already been quoted.

In Figure 1 the summer-wood width has been plotted over ring width for the breast-high sections of longleaf pine with a density of 350 trees per acre. If all of the points are considered, the line around which they group is determined by the equation $y = -0.05 + 0.652x - 0.041x^2$. In other words, it is a parabola which is concave downward. In this equation y represents the width of the summer wood and x the width of the annual ring.

The general relationship established above for the breast-high sections likewise holds for all of the heights studied in the same trees and for the breast-high sections of longleaf pines from the same tract, but which were grown under different degrees of stocking in the stand. The equations for these other sections are presented in Table 1, and the curves in Figure 2. The relation between summer-

wood width and ring width in loblolly pines from eastern North Carolina and Virginia is expressed by similar curves. (Fig. 3.) Thus it

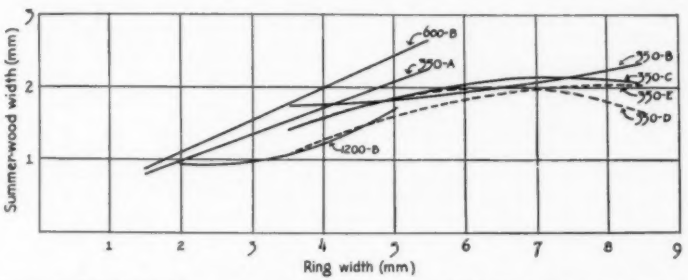

FIGURE 2.—Relationship between summer-wood width and ring width in longleaf pine when all rings on any cross section are included. In the curve designations, the number indicates the number of trees per acre in the stand, and the letter the height above ground, as follows: A, Stump height; B, 4½ feet; C, 8½ feet; D, 12½ feet; E, 16½ feet

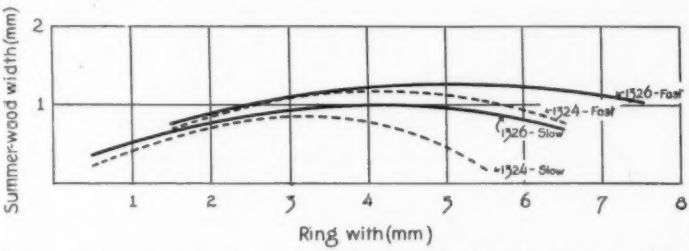

FIGURE 3.—Relationship between summer-wood width and ring width in loblolly pine, when all rings on the cross section 16 feet above ground are included. No. 1326 from Windsor, N. C., and No. 1524 from Franklin, Va. "Fast" indicates light stocking in the stand, and "slow" indicates heavy stocking

is to be expected that a parabola which is concave downward might express the relationship at any height in the southern pines.

TABLE 1.—*Equations expressing the relationships between ring width and the spring-wood width, summer-wood width, and percentage of summer wood*

Designation [a]	Height above ground	Equations for width of all rings, over ring width		Equations for width of all but 8 inner rings, over ring width	
		Spring wood	Summer wood	Spring wood	Summer wood
	Feet				
350–A	1.5	$y=0.063+0.447x+0.011x^2$	$y=-0.084+0.64x-0.031x^2$	$y=0.45x+0.08$	$y=0.50x+0.07$
350–B	4.5	$y=.232+.265x+.047x^2$	$y=-.05+.652x-.041x^2$	$y=.55x-.13$	$y=.42x+.23$
350–C	8.5	$y=.634+.091x+.07x^2$	$y=-.26+.7x-.049x^2$	$y=.50x+.17$	$y=.42x+.07$
350–D	12.5	$y=.35+.264x+.056x^2$	$y=-.24+.69x-.054x^2$	$y=.59x-.07$	$y=.36x+.19$
350–E	16.5	$y=-.354+.315x+.049x^2$	$y=-.06+.54x-.035x^2$	$y=.61x-.04$	$y=.33x+.21$
1200–B	4.5	$y=.27+.031x+.14x^2$	$y=.42+.65x-.086x^2$	$y=.54x-.09$	$y=.35x+.25$
600–B	4.5	$y=.305+.201x+.061x^2$	$y=.334+.371x-.003x^2$	$y=.45x+.09$	$y=.46x+.14$
1326–F	16	$y=.481+.205x+.079x^2$	$y=.22+.402x-.039x^2$	$y=.69x-.22$	$y=.27x+.285$
1326–S	16	$y=.45+.176x+.068x^2$	$y=.16+.409x-.05x^2$	$y=.64x-.1$	$y=.28x+.215$
1324–F	16	$y=.996-.071x+.115x^2$	$y=-.117+.642x-.078x^2$	$y=.65x-.08$	$y=.31x+.2$
1324–S	16	$y=-.08+.627x+.039x^2$	$y=-.03+.549x-.087x^2$	$y=.71x-.12$	$y=.13x+.38$

[a] Sections 350, 600, and 1,200 are of longleaf pine grown in Escambia County, Fla.; the section numbers indicate the number of trees per acre in each stand. Sections 1326 and 1324 are of loblolly pine, the former grown in Windsor, N. C., the latter in Franklin, Va.; designation "F" signifies fast-growing, lightly stocked stand; "S", slow-growing, heavily stocked stand.

TABLE 1.—*Equations expressing the relationships between ring width and the spring-wood width, summer-wood width, and percentage of summer wood*—Continued

Designation	Height above ground	Equations for width of inner 8 rings, over ring width		Equations for summer-wood percentage, over ring width
		Spring wood	Summer wood	
	Feet			
350–A	1.5	$y=0.57x+0.36$	$y=0.40x+0.15$	$y=52.83-0.288x-0.146x^2$
350–B	4.5	$y=-.17+.517x+0.027x^2$	$y=1.75-.045x+0.013x^2$	$y=55.26-.676x-.311x^2$
350–C	8.5	$y=1.39-.039x+.076x^2$	$y=.085+.6x-.041x^2$	$y=51.60-1.48x-.167x^2$
350–D	12.5	$y=.30+.36x+.045x^2$	$y=.60+.79x-.061x^2$	$y=50.00-1.309x-.239x^2$
350–E	16.5	$y=.09+.437x+.036x^2$	$y=-1.08+.78x-.049x^2$	$y=42.56+.212x-.305x^2$
1200–B	4.5	$y=1.86-1.19x+.362x^2$	$y=1.43-.467x+.103x^2$	$y=53.29+3.8x-2.06x^2$
600–B	4.5	$y=.61x+.07$	$y=.46x+.15$	$y=51.03+1.724x-.761x^2$
1326–F	16	$y=2.883+1.513x-.037x^2$	$y=2.106-.37x+.034x^2$	$y=45.12-.69x-.559x^2$
1326–S	16	$y=1.07x-2.39$	$y=.01x+.91$	$y=37.81+10.47x-3.637x^2$
1324–F	16	$y=.96x-.6$	$y=.07x+.44$	$y=41.36+.034-.546x^2$
1324–S	16	$y=.90x-.3$	$y=.09x+.39$	$y=39.03+.73x-1.214x^2$

While plotting the points, however, it was noticed that in the rings near the center of the trees the relationship between the two variables changed, the points for the inner rings falling below the line established by the rings nearer the periphery of the tree. This departure began somewhere between the fifth and tenth rings from the pith, and varied in different trees. After several trials a dividing line was

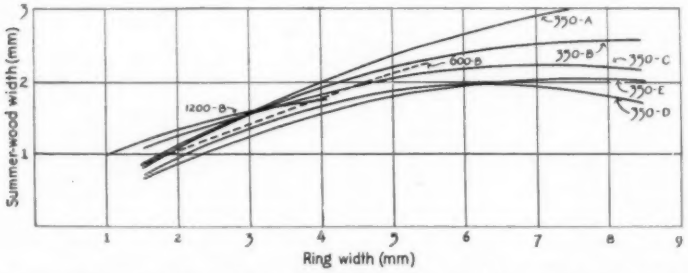

FIGURE 4.—Relationship between summer-wood width and ring width in longleaf pine when only the inner eight rings on any cross section are included. Curve designations as in Figure 2

arbitrarily established between the eighth and ninth rings, and this was used throughout the investigation.

If the curve delimited by the points for the eight inner rings (designated by circles in fig. 1) be determined, it is found to be the parabola $y=1.75-0.045x+0.013x^2$. These data are nearly as well satisfied by the straight line $y=0.07x+1.64$; since the algebraic sum of the squares of the residuals is -0.0267 for this curve, and -0.0124 for the parabola. An examination of the trend of points for this group of rings will show that, theoretically at least, it is more nearly expressed by a parabola than by a straight line. It is conceivable, however, that the fewer the wide rings the less reliable are the points at the right-hand end of the curve, and the greater their tendency to be satisfied by the equation for a straight line. This explains why this type of equation adequately satisfies the condition in some of the curves discussed later. Figure 4 shows the trend of the eight inner rings for each of the longleaf pine sections.

Figure 1 shows that the relation between the width of the summer wood and the width of the ring, for the rings toward the outside

of the tree, is represented by a straight line with a marked upward slope. In this case the line is $y=0.42x+0.23$. This does not agree with the statement made by Forsaith (*4*) since, if the summer-wood width remained constant, this line should have a slope of zero, i. e., should be parallel to the horizontal axis. Table 1 gives the equations for the same relation in the other longleaf and loblolly pine sections studied. The curves for the longleaf pine sections are presented in Figure 5. In every case studied, the relation is expressed by a similar straight line.

An interesting relation between the slope of these curves is observable. The slope is expressed by the constant accompanying the x in the equation. If the line were parallel to the x axis, i. e., horizontal, the constant would be zero; if the line were parallel to the y axis, the constant would be unity. In the same trees, the slope decreases as the distance aboveground increases, except that the breast-high section shows a slope which approximates that at the 8½-foot level. It will be noted later that the breast-high section attained a higher

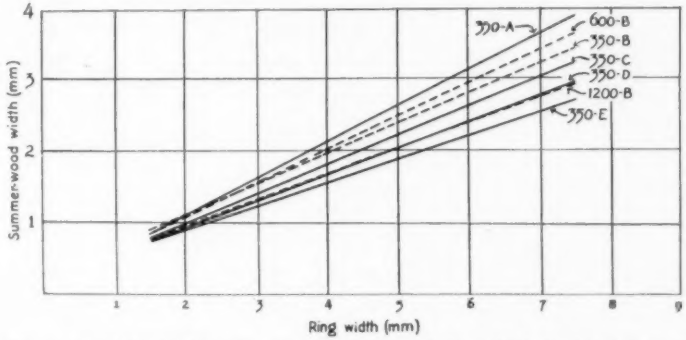

FIGURE 5.—Relationship between summer-wood width and ring width in longleaf pine for all except the inner eight rings on any cross section. Curve designations as in Figure 2

maximum percentage of summer wood than did the 8½-foot level. As the density of stocking increases in the stand the slope of the summer-wood-ring width curve increases until the density of 600 trees per acre is reached and then decreases as the number of trees per acre becomes greater.

When it was noted from the foregoing results that the age of the ring entered as one of the variables, 3-plane drawings for each section were made. The data for these were not sufficient to justify the calculation of equations, and only two of the diagrams are presented. Figure 6 shows graphically the relationship between ring width and summer-wood width, as correlated to the number of rings from the center of the tree. The curves representing the relationship between summer-wood width and number of rings from the pith have been smoothed by estimation; those running in the other direction have not been adjusted. These diagrams show the parabolic relationship between ring width and summer-wood width. They also indicate that the curvature in this relationship increases as the rings become wider. At any one age in a given cross section of the trunk it is evident that the summer-wood width increases with ring width. This gives the straight-line relationship already discussed.

RELATION BETWEEN SPRING-WOOD WIDTH AND RING WIDTH

Since the spring-wood zone is but the difference between the total ring width and the width of the summer wood, the curves representing the relationship between spring-wood width and ring width could be

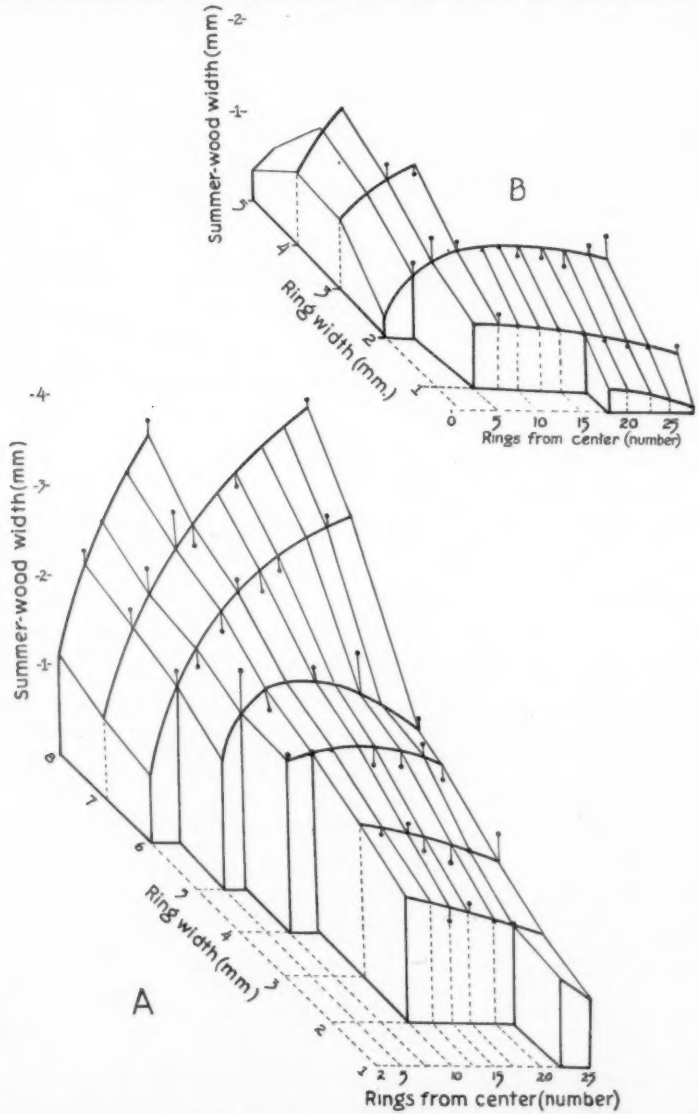

FIGURE 6.—Relationship between ring width and summer-wood width in longleaf pine at different distances from the center of the tree: A, At breast height in a stand with 350 trees per acre; B, at breast height in a stand with 1,200 trees per acre

obtained by subtracting the summer-wood width as read from the curves from the ring width. These data have been computed separately, however, in the same manner as were those for the summer wood, and the equations are presented in Table 1.

As was to be expected, the relation between spring wood and ring width, when all rings on one cross section of the trunk are considered, is expressed by a parabola which is concave upward; when only the eight inner rings are considered, the curve is either a straight line or a parabola; as the rings between the periphery and the eighth ring from the pith become wider, the curve is a straight line with a marked positive slope, i. e., an upward trend; and as the distance aboveground becomes greater, the slopes of the curves for the outer rings increase, except at the 4½-foot level.

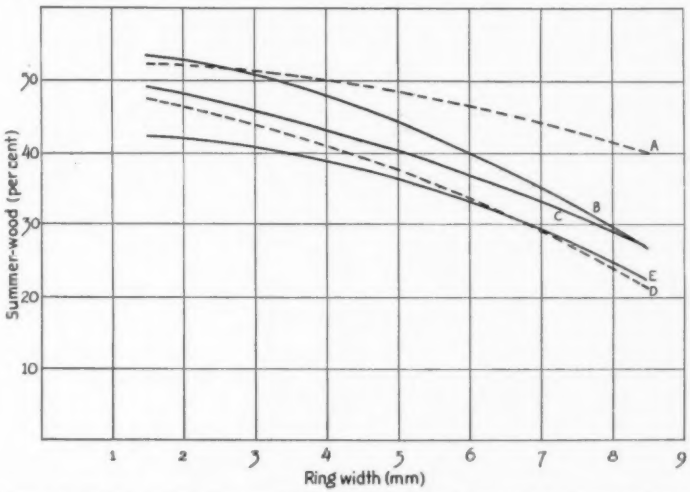

FIGURE 7.—Relationship between percentage of summer wood and ring width at different heights in longleaf pine grown in a stand containing 350 trees per acre. Curve designations as in Figure 2

RELATION BETWEEN PERCENTAGE OF SUMMER WOOD AND RING WIDTH

The points obtained by plotting the percentage of summer wood in each annual ring over the width of that ring tend to group around a parabolic curve which is concave downward. This form of curve, as illustrated in Figures 7, 8, and 9 holds throughout all sections of all the pines studied, being modified in the degree of curvature and the maximum percentage attained by both distance above ground and by density of stocking in the stand.

It is possible to vary the degree of curvature of the curves representing the relationship between summer-wood percentage and ring width, by including the wider rings, or, in other words, a larger proportion of the inner rings of any cross section. As the proportion of rings over 10 mm in width becomes greater, the curvature decreases until the data are best satisfied by a straight line with a negative slope, or even by a parabola which is slightly concave upwards. The wide inner rings were so few in number in the trees studied that the data

were deemed unreliable, and in no case have rings over 9 mm in width been used. In several instances, notably in the case of slow-

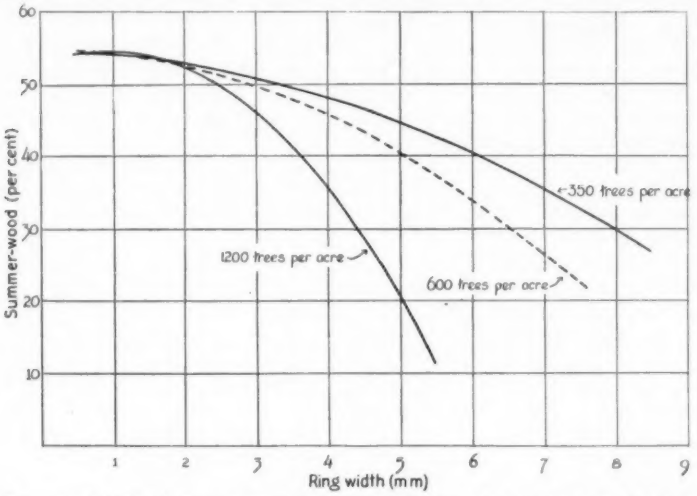

FIGURE 8.—Relationship between percentage of summer wood and ring width at breast height in longleaf pine from stands of different density

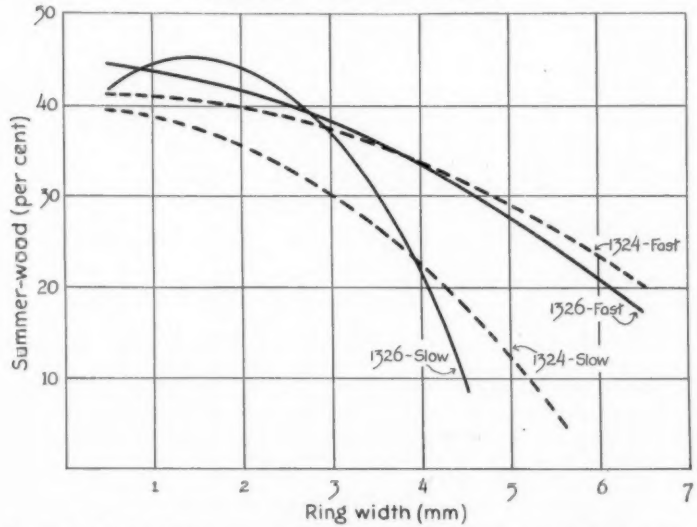

FIGURE 9.—Relationship between percentage of summer wood and ring width at 16 feet above-ground in loblolly pine from stands of light and heavy stocking. "Fast" indicates light stocking and "slow", indicates heavy stocking

growing trees, the maximum reliable ring width was found to be less than 9 mm.

The points have a rather wide dispersion in all the material studied. This can be accounted for only through the variations introduced by

the effect of growth factors on the separate parts of the ring. From the results reported previously by the writer (*11*) it is conceivable that an annual ring of any given width on any cross section of the tree might evince differences in the percentages of summerwood, depending upon the distribution of the rainfall during the vegetative season. Summer rainfall below the average in total quantity or at infrequent intervals is very likely to result in a smaller percentage of summer wood than if the summer rainfall is normal. Some evidence in support of this was noted while the points for the curves were being plotted. The values for certain years fell rather consistently above or below the average for all years. The writer has also indicated previously that individual trees respond qualitatively to precipitation according to their vigor. Because of irregularities arising from these causes, it is necessary to consider a number of trees, and the greater the number of trees the more accurate the picture presented by the conclusions.

EFFECT OF DISTANCE FROM GROUND

The effect of distance from the ground in the same individual was investigated in longleaf pine with a density of 350 trees per acre, since this was the only group for which material at different heights was available. These trees, it will be recalled, were selected from a stand in Escambia County, Fla. Sections were available at stump and breast height and at intervals of 4 feet above the latter point up to 16½ feet. The curves expressing the relationship between percentage of summer wood and ring width for these heights are presented in Figure 7.

EFFECT OF DENSITY OF STOCKING

The effect of density of stocking of the stand on the relation of percentage of summer wood to ring width was likewise investigated in the longleaf pine from Escambia County. From curves for the breast-high sections from trees representing the different stand densities, as presented in Figure 8, it is evident (1) that within the limits studied the trees from the denser stands attain a maximum percentage of summer wood nearly 3 per cent greater than do trees from less dense stands; (2) all stands present their maximum density in rings 0.5 mm in width; and (3) the open stands maintain a maximum percentage of summer wood through wider rings than those in which it is found in denser stands. The only other southern pine material available for similar studies was loblolly pine from Franklin, Va., and from Windsor, N. C. Both of these samples were limited in the number of trees represented, as only those of largest and smallest diameter were selected. The former were considered as growing under less crowded conditions than the latter. The curves expressing the relationship between percentage of summer wood and ring width at approximately 16 feet above the ground are presented in Figure 9. Thus in loblolly pine, crowding in the stand exerts the same influence on this relationship as it does in longleaf. The influence appears to be the same whether the trees grew in Florida or in the coastal plain of North Carolina and Virginia. Figure 9 shows also that the relationships which were noted for longleaf pine at breast height hold in their essentials for other heights in the bole of loblolly pine. The densely crowded trees from North Carolina (No. 1326) possessed narrower annual rings than any of the other groups studied. In these trees the

maximum percentage of summer wood was obtained in rings approximating 1.5 mm in width, rather than in rings 0.5 mm or less as in trees of faster growth. The general trend of the curves in Figures 8 and 9 leads to the conclusion that the slower the rate of growth, the wider the rings in which the maximum percentage of summer wood is reached, and the more rapid the decrease in the percentage of summer wood in rings narrower or wider than the point at which the maximum is attained.

A study of Figures 7, 8, and 9 shows that there is no universal relationship between ring width and the percentage of summer wood. The precentage of this thick-walled portion in a ring of any given width depends partly upon the portion of the tree in which this was formed and partly upon the amount of growing space available. That it is also dependent upon site conditions is likewise probable, though the material available during this study did not permit of such an investigation. Keinholz (*8*) has shown that the average percentage of summer wood in the first 60 rings of lodgepole pine (*Pinus contorta* Loud.) varied with the site. Trees from bogs possessed a maximum of 44.8 per cent summer wood, from virgin growth on good sites 28.5 per cent, and from lava sites a maximum of 22 per cent. Paul (*13*), as noted earlier in this paper, decided that on better sites the specific gravity of the wood was generally higher.

DISCUSSION

One of the outstanding facts all through this investigation was that the general relationships between the two portions of any annual ring and the width of the ring itself hold as well for second-growth loblolly pine as for second-growth longleaf. They also hold whether the loblolly was grown in Virginia or in eastern North Carolina. Preliminary investigations on pitch pine (*Pinus rigida* Mill.) and scrub pine (*P. virginiana* Mill.) from elevations of 2,200 feet in the mountains of southwestern Virginia, indicate identical results. These lend support to the view that the relations here established will be found to hold true generally for second-growth southern pines.

In all of the material studied the inner rings departed noticeably from the relationship established by the rings nearer the periphery of the cross section. As has been previously mentioned, this departure appeared somewhere between the fifth and tenth rings from the pith. It is apparently specific to the individual tree, and does not depend upon distance aboveground or upon amount of growing space. Neither is it caused by the greater average ring width in the inner rings. Figures 3 and 4 show that, where rings of the same width occur in the outer and inner zones of the cross section, the summer wood is almost invariably smaller in proportion in the inner rings. Similarly, as the pith is more closely approached, rings of the same width as found further toward the periphery possess a smaller percentage of the thick-walled summer wood.

The data do not indicate the reason for this departure, but it may be explained eventually on the basis of different metabolic conditions in young stems. The departure from the normal of later life is such as to result in a decreased percentage of summer wood.

It has been shown how the lower percentage of summer wood in the inner rings influences the trend over the cross section as a whole. Whether the straight-line tendency of the relation in the outer rings

between summer wood and ring width and consequently between spring wood and ring width would be maintained if a larger number of wide rings were available in some sections and a greater number of narrow rings in others is problematical. The fact that these curves do not reach a zero summer-wood width when the ring width is zero indicates that the curvature might be modified if a greater number of narrow rings were present. The trend of the points which are so few as to have been deemed unreliable in computing the equations is such as to indicate a probable parabolic tendency. Be that as it may, the reliable data available are best satisfied by straight lines.

From the mathematical standpoint, each of the parabolas expressing the relationship between summer-wood width and ring width may be considered as the resultant of two curves. The first of these is a straight line with a positive slope; the second is a parabola with its vortex at the origin and lying wholly below the x axis. Since the normal relation between summer-wood width and ring width appears to be expressed by a straight line with a positive slope, the first of these two curves might well be considered as expressing the normal relationship. The second would then be considered as representing the factors which tend to prevent the formation of summer wood. These latter factors exert insufficient control to affect the normal tendency in narrow rings, but become more marked in wide rings and especially in the wide rings near the center of the tree. Further controlled experiments may eventually permit these factors to be determined.

SUMMARY

A study of approximately 10,000 rings on second-growth longleaf and loblolly pines from Florida, North Carolina, and Virginia has shown some interesting relationships between the ring width and the percentage of summer wood. The material available has been of such a nature as to permit a study of these relationships under different conditions of stand density, and at different heights in the same individual trees. The following points are deemed to have been established for the second-growth stands studied:

On any cross section the relationship between the percentage of summer wood and the ring width may be expressed by a parabola which is concave downward.

The maximum percentage of summer wood attained at stump height is approximately that attained at breast height in the stand containing 350 trees per acre.

The decrease in the maximum percentage of summer wood attained appears to be more rapid in the uppermost portion of the bole.

The wide rings contain a higher percentage of summer wood at points near the ground.

The maximum percentage of summer wood attained at a given height in the tree appears to be independent of the density of stocking.

The maximum percentage of summer wood at a given height in the bole occurs only in the narrowest rings in crowded stands, but in more open stands this maximum may be maintained in wider rings.

The relationship between summer-wood width and ring width on any cross section is expressed by a parabola which is concave downward when all rings between the pith and the periphery are considered. Conversely, the relationship between spring-wood width

and ring width in the same material is expressed by a parabola which is concave upward.

In the outer rings of a given cross section, i. e., outside the eighth ring, the relationship between summer-wood width and ring width is expressed by a straight line with a positive slope. This is also true for the spring wood.

The relationship between either spring-wood or summer-wood width and ring width in the inner eight rings is variable. It may sometimes be expressed by a straight line and sometimes by a parabola.

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THE HEMOGLOBIN CONTENT OF THE BLOOD OF HEALTHY AND ANEMIC "SALT-SICK" CATTLE[1]

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INTRODUCTION

A condition known as "salt sick" affects cattle kept too long on certain range areas in Florida. Loss of appetite, followed by progressive loss of condition even to the point of emaciation, are the most prominent symptoms. Recent investigations (*4*)[3] have proved this to be a nutritional anemia. The concentration of hemoglobin in the blood of cattle has been studied as an index of the development of or recovery from this condition. Limited data are available concerning the average percentage of hemoglobin in bovine blood, but such variations as might be expected, considering the behavior of the other blood constituents, have never been measured for cattle under uniform conditions of feeding and management. Aston (*2*), by some iron determinations on the blood of "bush sick" (iron deficient) cattle in New Zealand, has shown anemia to be one of the symptoms of that condition. No direct determinations of hemoglobin were made.

The object of this study was to determine the variations in the hemoglobin content of the blood of cattle observed at 2½-hour, daily, and semiannual intervals, the average values for healthy cattle, and the variations from these values in an anemia due to a naturally occurring iron, or iron and copper, deficiency, such as occurs in salt-sick cattle.

METHODS OF EXPERIMENTATION

Hemoglobin determinations at the Florida experiment station were made in duplicate by the Newcomer (*11*) acid-hematin method on blood drawn from the jugular vein. The glass color standard manufactured by Bausch & Lomb was used. Samples were collected in small glass vials containing just sufficient dry sodium citrate to prevent clotting. Analyses were made immediately.

Determinations in field studies were made with a standardized Dare hemoglobinometer (*7*) on blood obtained by puncturing the marginal ear vein. The same operator made all of the readings reported. All results have been converted to grams of hemoglobin per 100 ml of blood.

It is recognized that the Dare instrument is not so accurate for individual analyses, but in a series of samples the average value

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[2] Grateful acknowledgment is made to tick-eradication officials, Smith-Hughes teachers, and county agents for their assistance in establishing contacts with owners of "salt-sick" cattle; to Dr. C. H. Eckles for permission to obtain data from the Minnesota experimental station herd; to the managers of Florida State institutional herds for cooperation in the study of healthy cattle; and to J. R. Greenman for technical assistance.

[3] Reference is made by number (italic) to Literature Cited, p. 563.

obtained by the Dare method very closely approximates that obtained by the Newcomer method. It is felt that averages of series by either of the two methods can be compared.

EXPERIMENTAL DATA

VARIATIONS IN BLOOD HEMOGLOBIN CONTENT AT 2½-HOUR INTERVALS

Ten Jersey heifers and dry cows were used in this study, the first blood samples being taken at 6 a. m., and successive ones at 2½-hour intervals until 9 p. m. The data are presented in Table 1.

TABLE 1.—*Hemoglobin determinations (Newcomer method) on blood samples taken at 2½ hour intervals from Jersey cattle in 1930*

Date and animal No.	Grams of hemoglobin per 100 cc of blood at indicated time of taking sample								Standard deviation	Coefficient of variation
	6 a. m.	8.30 a. m.	11 a. m.	1.30 p. m.	4 p. m.	6.30 p. m.	9 p. m.	Average		
Sept. 3:										
395	6.71	6.22	6.44	6.68	6.04	6.68	6.44	6.46	0.237	3.67
401	8.18	9.40	9.05	9.60	9.36	10.24	9.26	9.30	.574	6.17
410	8.81	8.90	9.87	9.53	9.67	9.60	9.57	9.42	.373	3.96
Sept. 9:										
295	15.90	15.60	15.43	16.23	15.25	16.46	16.12	15.86	.412	2.60
312	16.46	15.52	15.34	16.34	15.69	14.73	16.23	15.76	.579	3.67
315	14.64	12.98	14.12	14.90	14.38	13.75	15.43	14.31	.740	5.17
334	15.52	14.82	14.82	16.46	16.01	15.34	16.12	15.58	.594	3.81
Sept. 11:										
188	15.16	13.33	14.38	14.20	14.03	13.89	14.20	14.17	.511	3.61
300	12.05	12.91	12.22	12.84	13.61	13.00	12.40	12.72	.496	3.90
305	13.47	13.40	13.47	13.33	13.61	13.33	13.68	13.47	.124	.92
Sept. 12:										
295	14.90	15.25	15.34	15.25	14.90	15.25	16.12	15.29	.378	2.47
312	16.57	14.64	15.08	15.90	14.82	14.99	14.73	15.25	.664	4.35
315	13.40	13.75	14.38	14.20	15.16	14.38	12.57	13.98	.769	5.50
334	14.20	14.29	15.08	13.89	13.75	14.20	14.20	14.23	.391	2.75
Average	13.28	12.93	13.21	13.52	13.30	13.27	13.36	13.27	.162	1.22

Animals No. 395, 401, and 410, were kept in dry lot with water available at all times. The usual ration of silage, hay, and mixed grain was fed. The other animals were on a small Bahia-grass pasture without supplemental feed but with water continuously available. The effect of different methods of feeding should thus be eliminated.

One purpose of this study was to determine whether or not any of the observed variations could be avoided by obtaining the blood samples at some particular time during the day. Assuming that the mean of seven determinations per animal per day represents the value for that day, it would appear from the correlation coefficients between the mean, and the several series of samples, that the series taken at 1.30 p. m. most nearly parallels the mean under the conditions encountered. The next most satisfactory time was 8.30 a. m. The correlation coefficients for the two times were $+0.9941 \pm 0.0033$ and $+0.9890 \pm 0.0039$, respectively.

Apparently there is a tendency for determinations made on early-afternoon samples to give higher results than those obtained on early-forenoon samples. However, the average of the standard deviations for the 14 animals (10 individuals), was ± 0.489 g and the standard deviation of the means was ± 0.162 g. This indicates that the variations due to individuality are much greater than those due to time of sampling, and also that individual variations tend to balance each other.

VARIATIONS IN BLOOD HEMOGLOBIN CONTENT FROM DAY TO DAY

A number of the heifers in the young herd kept in dry lot were bled over periods of 7 to 30 days. Blood samples were taken each morning between 8.30 and 9 o'clock to avoid the possibility of complications due to the time of sampling. The first three animals were under observation during the time of tick eradication [5] and no blood samples were taken on the days when the cattle were dipped. Feeding and management were uniform at all times. The results of this study are presented in Table 2.

No uniformity could be detected in the variations observed. Changes were irregular and no cycles were evident. Several high or low values might, or might not, occur in succession so that day-to-day variations can not be eliminated entirely by taking an average of several successive analyses. The greatest coefficient of variation observed was 9.18 per cent and the average was 5.28 per cent. This variation, as well as that observed during the day, must be considered in the interpretation of the significance of the hemoglobin concentration in bovine blood from time to time.

TABLE 2.—*Variations in hemoglobin content of bovine blood from day to day when animals were bled 7, 10, and 30 days consecutively*

Animal No.	Hemoglobin per 100 cc blood			Coefficient of variation
	Range	Mean	Standard deviation	
	Grams	*Grams*	*Gram*	*Per cent*
ANIMALS BLED 7 CONSECUTIVE DAYS, BEGINNING JUNE 23, 1930				
358	12.91–14.46	13.65	0.55	4.03
380	9.79–10.86	10.18	.37	3.63
382	10.62–12.05	11.42	.53	4.64
392	9.02–10.20	9.40	.37	3.94
401	10.52–11.48	11.06	.35	3.16
405	10.11–11.71	11.15	.49	4.39
408	9.16– 9.79	9.45	.24	2.54
410	7.94– 8.90	8.39	.35	4.17
419	8.05– 8.96	8.64	.28	3.24
423	7.57– 8.05	7.71	.17	2.20
431	8.37–10.52	9.78	.72	7.36
434	8.21–10.11	9.13	.59	6.46
ANIMALS BLED 10 CONSECUTIVE DAYS, BEGINNING SEPTEMBER 9, 1931				
358	12.51–14.03	13.30	0.49	3.68
380	9.30–11.19	10.18	.58	5.70
382	10.11–11.48	11.01	.36	3.27
392	9.23–11.59	10.10	.78	7.72
401	9.09–11.10	10.10	.65	6.44
405	9.43–12.17	10.70	.68	6.36
408	7.66– 8.90	8.14	.36	4.42
410	8.12–10.95	9.15	.84	9.18
423	7.47– 8.96	7.98	.36	4.51
431	7.52– 9.57	8.59	.63	7.33
434	8.12–10.90	9.33	.75	8.04
435	9.43–12.28	10.35	.78	7.54
ANIMALS BLED 30 CONSECUTIVE DAYS, BEGINNING SEPTEMBER 3, 1930				
395	6.13–8.86	7.04	0.47	6.68
401	7.66–9.68	8.64	.61	7.06
410	8.28–9.99	9.05	.44	4.86
Average				5.28

[5] All cattle in this area were dipped in an arsenical solution at 14-day intervals, in accordance with State and Federal regulations for the eradication of the cattle-fever tick, *Margaropus annulatus*.

VARIATIONS IN BLOOD HEMOGLOBIN CONTENT WITHIN A HERD AT SEMIANNUAL INTERVALS

Blood samples were taken from all cattle in the experiment station herd semiannually to test for contagious abortion. Hemoglobin determinations were made at these times on the blood of 21 cows at each of the last four intervals, other cows being eliminated from the tabulation because they were not bled every time. Feeding and management were uniform throughout these years. The average hemoglobin values for the 21 head are as follows:

	Grams of hemoglobin per 100 cc of blood
September, 1930	9.77
March, 1931	12.34
September, 1931	11.00
March, 1932	10.12
Average	11.31

The highest single determination was 15.25 g, and the lowest 7.92 g. The least variation for an individual for the four bleedings was 0.48 g, and the greatest 6.59 g. Based on the averages for the particular individuals, these variations are equivalent to 5.16 and 64.80 per cent, respectively.

VARIATIONS IN BLOOD HEMOGLOBIN CONTENT OF NONSALT-SICK CATTLE

To supplement the data in the literature relative to the average concentration of hemoglobin in bovine blood, and to secure data from cattle kept under conditions similar to those encountered in the investigation of a naturally occurring nutritional anemia in Florida, hemoglobin determinations were made on blood samples obtained from two of the State institutional dairy herds in west Florida. Other determinations were made at the Minnesota experiment station, and in some privately owned commercial herds in western Pennsylvania. The numerous determinations of McCay (*9*) had not then been reported. The data secured, together with a résumé of that in the literature, are presented in Table 3.

TABLE 3.—*Average hemoglobin content of bovine blood from areas where nutritional anemia ("salt sick") is unknown*

State and county	Investigators	Kind of cattle	Analyses	Hemoglobin per 100 cc of blood			Method used
				Range	Mean	Standard deviation	
			Number	*Grams*	*Grams*	*Grams*	
Florida, Jackson, and Leon counties.		Cows	20	10.99–16.49	14.61	1.45	Dare (*7*).
		do	52	10.99–16.49	14.12	1.32	Do.
		do	60	9.09–14.20	11.06	1.40	Newcomer (*11*).
Minnesota, Ramsey County.		Heifers	7	11.40–16.49	14.58	1.68	Dare.
Pennsylvania, Lawrence County.		Cows	39	10.99–16.49	12.68	1.36	Do.
		Heifers	4	12.37–15.11	13.47		Do.
		Calves	3	11.68–15.39	14.06		Do.
Kansas	Brooks and Hughes (*5*).	Cows	103		10.94	1.54	Newcomer.
		Heifers	59		11.71	1.24	Do.
		Calves	111		10.46	1.65	Do.
		Bulls	24		11.92	1.89	Do.
New York	Hayden and Fish (*8*).	Cows			14.10		Tallquist.
	McCay (*9*)	Cows and heifers.	1,073		10.90	.86	Cohen and Smith (*6*).
Pennsylvania	Anderson, Gayley, and Pratt (*1*).	Cows	5	11.19–15.04	13.19		Van Slyke (*12*).
		Calves	24	9.70–16.79	12.23		Do.

Values for mature cows using the data of Brooks and Hughes (*5*), McCay (*9*), and those presented in this paper, vary from 10.94 to 14.61 g of hemoglobin per 100 cc of blood. Average analyses by the Newcomer (*11*) and Cohen and Smith (*6*) methods vary from 10.94+1.54 to 11.06+1.40 g per 100 cc of blood. The limited data do not permit one to set a similar standard for younger cattle. The calves may or may not have a higher concentration of hemoglobin in their blood than the cows. The heifers, between 1 and 2 years of age, seem to show a higher concentration.

Definite information for young cattle can be secured only by regular periodic determinations of their blood hemoglobin from birth to maturity. The data tabulated make it appear doubtful whether as definite an age trend would be secured as has been given for man. However, these data are sufficient to emphasize the low values sometimes encountered in a naturally occurring iron, or iron and copper, deficiency.

EFFECT OF FEEDING IRON AND COPPER SUPPLEMENTS TO SALT-SICK CATTLE

Early in the investigation of salt sick it was noted that the blood was pale in color, and, when centrifuged, usually had less than half of the normal proportion of red corpuscles. The range cattleman's method of curing the condition was to remove his cattle to a different range. Observation showed that healthy ranges were on clay, had a clay subsoil, or were subject to overflow from clay land, while the affected ranges were on white or gray sands, marl soils, or muck lands. There was sufficient forage for the cattle on either type of range, so that quantity of feed available was not a factor.

The similarity of the physical symptoms with those reported for bush sickness (an iron starvation) by Aston (*3*) in New Zealand, the anemic condition of the cattle themselves, and the apparent scarcity of iron in the soils of the affected ranges, indicated that salt sick might be a form of iron starvation.

TABLE 4.—*Hemoglobin content (Dare method) of freshly drawn blood from cattle on iron, or iron and copper, deficient pastures*

Age data	Animals	Hemoglobin per 100 cc blood		
		Range	Mean	Standard deviation
	Number	*Grams*	*Grams*	*Grams*
HEALTHY CATTLE				
Cattle	2	9.62–13.74	11.68	
Yearlings	28	10.72–16.49	13.34	1.37
Over 2 years	4	11.68–14.43	13.57	
SALT-SICK CATTLE IN SAME HERDS				
Calves	12	3.02–9.62	5.92	1.62
Yearlings	96	3.44–13.05	8.38	2.05
Over 2 years	34	4.81–13.19	9.04	2.41
Over 12 years	2		4.81	
SALT-SICK CATTLE SHORTLY BEFORE DEATH				
Calves	9	1.37–9.62	4.49	2.89
Yearlings	7	3.44–12.37	6.62	2.58

Affected cattle were located in 39 herds distributed over the different soil areas where this nutritional anemia was found. Hemoglobin determinations were made on representative affected and healthy animals in these herds before supplemental minerals were provided for cooperative studies. The results of this survey are presented in Table 4.

The values obtained for the cattle which appeared physically fit in these herds, and which the owners considered to be healthy, compare favorably with the average values as given in Table 3. The affected animals had a lower concentration of hemoglobin in their blood than did the healthy animals, although a number of the affected animals would not have been suspected to be iron-deficient, as judged by the concentration of hemoglobin in their blood.

The lowest concentration of hemoglobin observed was 1.37 g per 100 cc in a calf which died, while 3.02 g was the lowest value for an animal that recovered.

The range of the analyses is much greater than is encountered in the normal groups. Apparently there are changes in the blood volume, or other physiological adaptations, that effect the concentration of hemoglobin in the blood stream to such an extent that even after allowing for normal variations it alone can not be regarded as an exact measure of anemic conditions. However, the average condition of a group of animals can be determined very closely by a study of their blood.

Owners of the affected herds were provided with ferric ammonium citrate. This was given as a drench at the rate of 2 or 3 ounces of a 6 per cent solution, according to the size and age of the animal. No other changes were made in feeding or management. Slow and irregular responses to the sole iron supplement suggested that some other element must be deficient also. Cattle at the Florida experiment station (*10*) made rapid and complete recovery from salt sick when copper sulphate was added to the iron compound in a 1 to 50 molecular ratio. This addition was then made to the supplement used in the field with uniformly favorable results.

Only a single hemoglobin determination was obtained on some of these cattle while as many as six or seven were made on others at approximately monthly intervals. The following tabulation shows the increase in the concentration of hemoglobin in the blood from the time of providing supplement until physical symptoms had almost completely disappeared:

	Grams of hemoglobin per 100 cc of blood
87 head, visibly affected	8.28
87 head, given supplement 1 month	10.61
79 head, near recovery	11.55

SUMMARY

Coefficients of variation up to 6.17 per cent were observed for the concentration of hemoglobin in bovine blood when the samples were taken at 2½-hour intervals. The concentration tended to be higher and more regular during the early afternoon and early forenoon. The coefficients of variation, based on 27 series of from 7 to 30 days, average 5.28 per cent. In the same herd under nearly uniform feeding and management, irregular changes in hemoglobin concentration were observed.

The average concentration of hemoglobin in the blood of cows determined by the more accurate methods, ranges from 10.94 ± 1.54 to 11.06 ± 1.40 g per 100 cc.

A study is reported of 39 herds on areas where cattle are affected with a naturally occurring nutritional anemia. Healthy animals in these herds have a normal concentration of hemoglobin in their blood. The lowest value observed in an anemic animal was 1.37 g per 100 cc in a calf shortly prior to death. Values as low as 3.02 g were observed in anemic cattle that recovered from the condition by the use of iron and copper supplement. A total of 79 animals given the above supplement without other change in feed or management regained a normal concentration of hemoglobin in their blood.

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NORMAL GROWTH OF POTATO LEAVES IN GREENHOUSE AND FIELD[1]

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INTRODUCTION

This paper records the growth of normal potato leaves in greenhouse and field during the early part of the summer, when optimum conditions for plant growth prevail. The immediate purpose of these studies was to ascertain the period of maximum surface expansion under normal conditions both during the day and during the night. An earlier study of the chloroplasts of the potato (*13*)[2] had shown that the period of maximum growth and division of the chloroplasts coincides with the period of maximum growth of the leaf. A search through the literature revealed few observations regarding the two problems here considered, namely, the size at which potato leaflets show maximum growth in area and the time (day or night) of maximun leaflet expansion.

REVIEW OF LITERATURE

The older work on leaf growth seems to have been stimulated largely by Sachs' theory of the period of maximum growth (*11*). Stebler (*12*) found that maximum growth took place in *Secale cereale* between noon and 3 p. m. and in *Allium cepa* between noon and 9 p. m., and that daily growth was greatest in the latter plant between the fifth and ninth days at 19° to 21° C. Kreusler's measurements of the area of maize foliage (*8*) indicated a period of accelerated growth at the appearance of tassels and ears. Wildt's measurements of clover leaves (*14*) led him to conclude that sunshine exerted more influence on the amount of actively functioning leaf surface produced than did temperature.

Gregory (*6*), studying the large, irregular, hexagonal to octagonal leaves of the cultivated cucumber, found that only a few of the leaves attained maximum growth at the same time. The rate of growth in a leaf, he believed, "tends to fall off, owing to the action of a detrimental factor."

No data on the growth of the potato leaf were found except those of Bunzel (*1*), who showed that a shoot continues to grow for 67 days, after which the weight of leaves and stalk becomes stationary. Lange (*9*), in a study of the development of chimeras in *Solanum lycopersicum* and *S. nigrum*, found nuclear and cell divisions most numerous between 3 and 5 a. m. In greenhouse plants on the night of March 7, 1925, the mitoses were few in number until 3 a. m.; at 4 a. m. a noticeable increase appeared; and at 5 a. m. the maximum number was present. These results correspond to those obtained by Lutman (*10*) and Karsten (*7*) in their studies of cell reproduction in certain of the algae. Such nuclear and cell divisions do not necessarily mean a marked increase in the bulk of an organ.

[1] Received for publication Dec. 7, 1931; issued April, 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 577.

In the mature leaves used by Denny (*4*) a gain in fresh weight and a loss in dry weight occurred during the night, indicating the absorption of water and the translocation of some of the materials, especially carbohydrates, and possibly nitrogenous compounds.

Davis and Sawyer (*3*) found sucrose present in the leaves in increasing amounts from sunrise to 2 p. m., but after that hour it decreased. Hexose sugars were also present, but the percentage varied owing to the formation or solution of the starch. From about 2 p. m. until late afternoon hexoses were abundant in the leaves, owing probably to conversion of the sucrose present. In the leafstalks reducing sugars predominated over sucrose. Storage of starch, hemicelluloses, sucrose, and dextrose are also recorded by Clements (*2*). He found the maximum at 6 to 8 p. m., with sucrose or dextrose present at all hours of the day and night, although not in very large quantities.

METHODS

The measurements herein recorded were made at the Vermont station on plants of Green Mountain potato (*Solanum tuberosum* L.) growing in pots in the greenhouse and in the open. No special effort was made to control temperature, humidity, or the length of day under which the plants were grown. Controlled experiments, while of scientific value, were entirely apart from the purpose for which these records were made. The temperature in the greenhouse never went below 60° F. at night but rose to above 100° in direct sunshine. The plants, in 5 to 6 inch pots containing rich greenhouse soil, were placed at night in a box electrically heated by resistance coils, which raised the night temperature in the box about 5°. The plants grown in the field were also in pots. The temperature, although the experiments were made in the latter part of July, fell to almost 50° on one of the nights. The plants were about 6 to 7 cm high when leaflet measurement began, and had five to six leaves. All branches except one were removed. The measurements of length and breadth were made with narrow strips of millimeter paper and were accurate to within about one-half millimeter. The very young leaflets open out in a fan-shaped figure, with the leaf lamina decurrent on the petiole, so that the measurements of length are not so accurate for the first two or three days after the leaflets appear as they are later. When the leaflets are 1 cm in length they flatten out and are as easy to measure as a piece of paper of the same size.

Certain selected leaves were measured as soon as they began to make rapid growth, the object in this case being not to trace the growth of the leaf but to determine the maximum increment of growth made during the day and during the night. Measurements made at 7 to 8 a. m. and 7 to 8 p. m. were plotted.

The leaves were reproduced to scale on coordinate paper, the daily or half-daily increments being added and a mature leaflet of the correct form and measurement built up. These areas were then determined with a planimeter. In view of the regular form of most of the potato leaflets this method should give results the error of which would not exceed 5 per cent.

GROWTH OF A SINGLE LEAF

The results of the measurement of a single typical leaf (sixth from the bottom, with four leaflets) from a greenhouse plant are shown in Figures 1 and 2. Leaflet A grew rapidly during the first eight days of recorded measurement, but growth declined after May 16, the daily

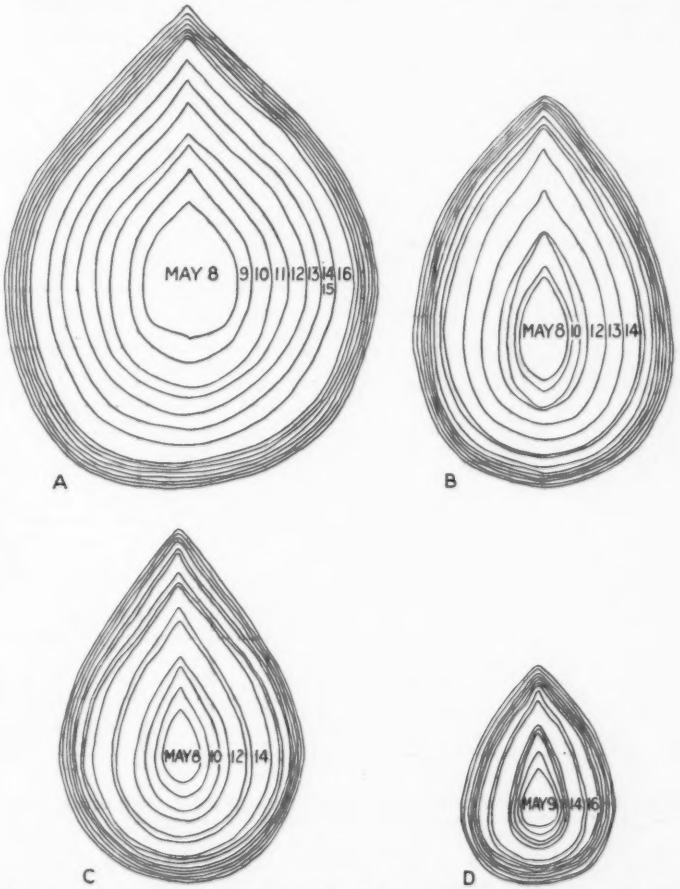

FIGURE 1.—Growth of the four leaflets on a typical leaf (No. 6 on the stem from the bottom), from May 8 to May 25, in the greenhouse

increase dropping abruptly from 23 per cent on May 15 to an average of 3 per cent for the remaining nine days. In leaflet B permanent slackening in growth occurred on May 17, the daily increase dropping from 15 per cent to an average of 3 per cent for the remaining eight days. Likewise, marked decrease in the rate of growth in leaflet C occurred on May 17, with a drop from 22 per cent to an average of 5 per cent. In leaflet D the more rapid rate of growth continued for a

slightly longer period, until May 20, when the rate of growth fell from 10 per cent to an average of 5 per cent for the remaining period of observation.

The curves of leaflet and of leaf growth (fig. 2) conform to the typical curve expressing the rate and distribution of growth in any of the other plant organs, such as stem or root. These curves of percentage increase were obtained by using the increase as the numerator and the area of the preceding day as the denominator of a

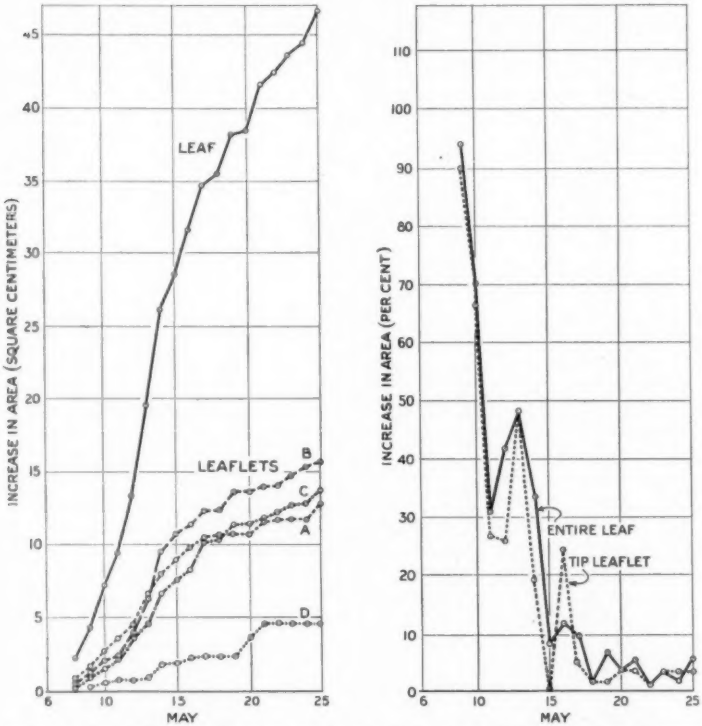

FIGURE 2.—Increase in area of entire leaf, and the four leaflets A to D, respectively, shown in Figure 1, and the percentage increase in the entire leaf and the tip leaflet

fraction. If the leaf was small and the increase in length and width was 1 mm, the percentage of growth was very much greater than if the leaf was much larger in area. Such a curve represents the proportionate growth more fairly than does a curve where the increment is simply added to a previous area. Such curves are given for the tip leaflet and for the entire leaf.

GROWTH OF LEAVES ON A SINGLE SHOOT

All leaves on three plants, each consisting of a single shoot, were measured between May 8 and May 25, 1931, in the manner described. Leaf 1 was nearest the base of the plant. By the end of the observa-

tion period one cluster of flower buds had appeared and a second had begun to form in the axil of one of the branches, the plant being then about 30 cm high.

The curves of growth of the leaves of the largest and sturdiest of the plants, as shown in Figure 3, indicate that while the basal leaf

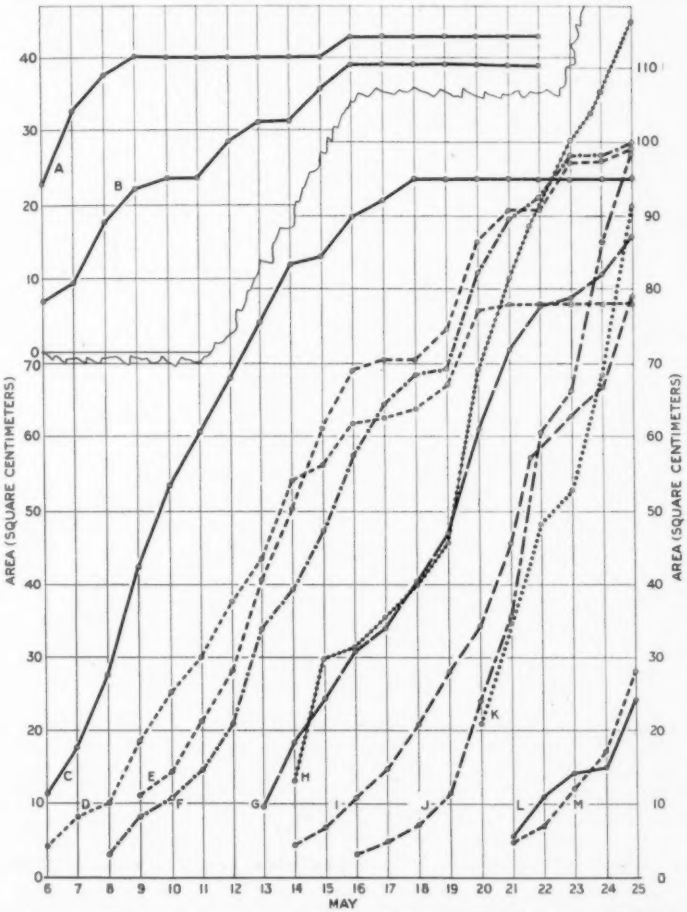

FIGURE 3.—Growth of all leaves on a shoot, leaf A being nearest the ground. Accelerated growth shifts to the right, i. e., up the stem, as new leaves are formed

grew very rapidly during the early days of the observations, this increase dropped off until its growth practically ceased. The period of accelerated growth on leaf 2 came a few days later, and growth continued over a longer period, but gradually it too came to a standstill. The growth of leaf 3 moved over still further toward the later period before growth slowed down, and the same was true of leaves 4, 5, and 6. Leaves 7 to 13 were still in the stage of rapid growth

when the observations were concluded on May 25. In fact, these leaves were so small that measurements could not be made on leaf 7 until May 13, and the first measurements on leaves 12 and 13 were not taken until May 21.

The five to seven leaflets which make up the compound leaf of the potato show almost simultaneously accelerated growth, although, as

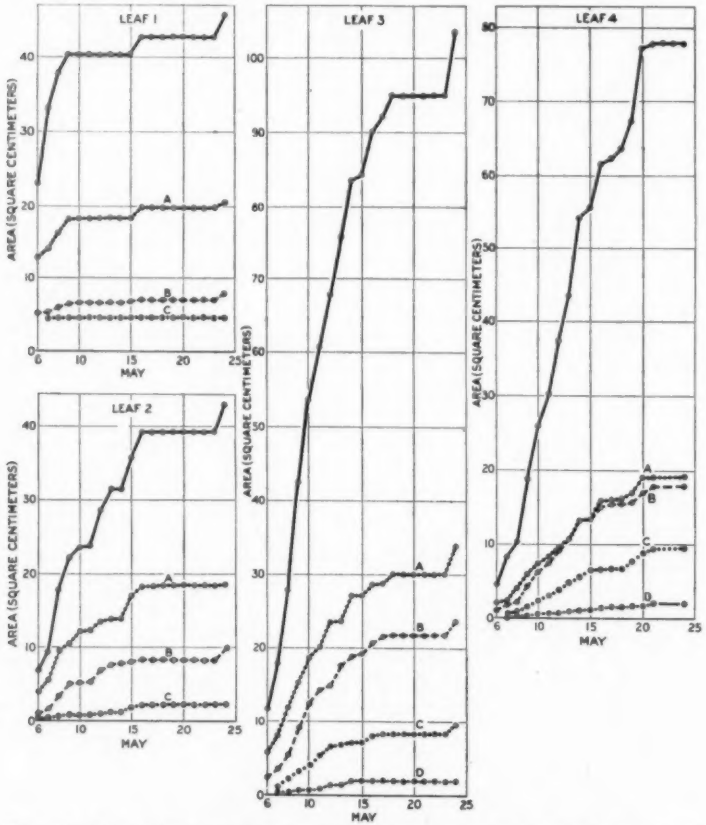

FIGURE 4.—Growth of representative leaves (solid lines) and their respective leaflets A–C and A–D

noted above, the period of most rapid growth moves slightly forward in point of time from the tip leaflet toward the base. (Fig. 4.)

The percentage of increase in the total leaf spread of three plants (fig. 5) indicates that, for a time after the plants are well above the surface of the ground, the rate of growth is very rapid. Then for a few days the increase is only 10 to 20 per cent each day, and thereafter it falls to an average of less than 10 per cent a day.

Almost identical curves were obtained from outdoor growth of leaves between July 17 and August 1. Some of the very small leaves

at the base of the plants were not measured. The leaves on the outdoor plants were larger than those on the greenhouse plants, but the slowing up of growth in older leaves and the beginning of growth in younger ones during the latter part of the observations were similar in both, although the outside temperature was never quite so high during the day as that in the greenhouse and several times at night sank below the minimum of the greenhouse.

Increase in leaf spread was continuous during the observations, as is indicated by the number of square centimeters of leaf. Flower

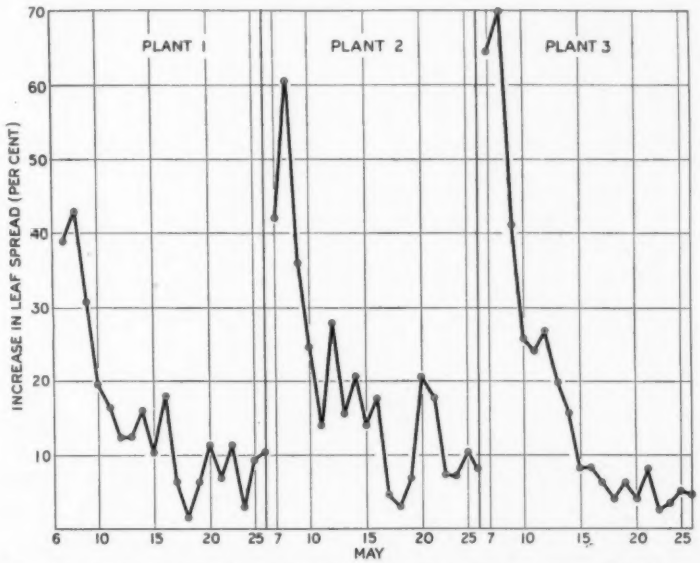

FIGURE 5.—Leaf growth of three typical plants from the greenhouse, shown as percentages of increase, the percentage being obtained by dividing the increment by the area on the preceding day

formation does not check vegetative growth, as little or no food materials flow into the flowers or seeds, the flowers being abortive attempts at seed formation. A cluster is formed at the top of the stem, but the shoot continues to grow and a new cluster is formed in the leaf axils. The young tubers are formed at about the same time, but do not draw heavily on the accumulated foods, as their accelerated growth takes place later.

DAY AND NIGHT GROWTH OF LEAVES ON GREENHOUSE PLANTS

The most rapid growth of leaflets occurred when they were about 1 cm in width and 1½ cm in length. This observation afforded a basis for determining the period during the day or night in which they grew most rapidly. Lange's observation (*9*) made on related species of Solanum, that the maximum number of cell divisions occurs at 5 a. m., was not of much assistance, for increase in number of cells may not be associated with growth in organ size. Cells grow to some

extent before they divide, but real increase in bulk comes after they start to grow by formation of vacuoles in the cytoplasm.

The leaflets selected showed variations in growth. (Table 1 and fig. 6.) In the greenhouse plants growth was fairly evenly distributed

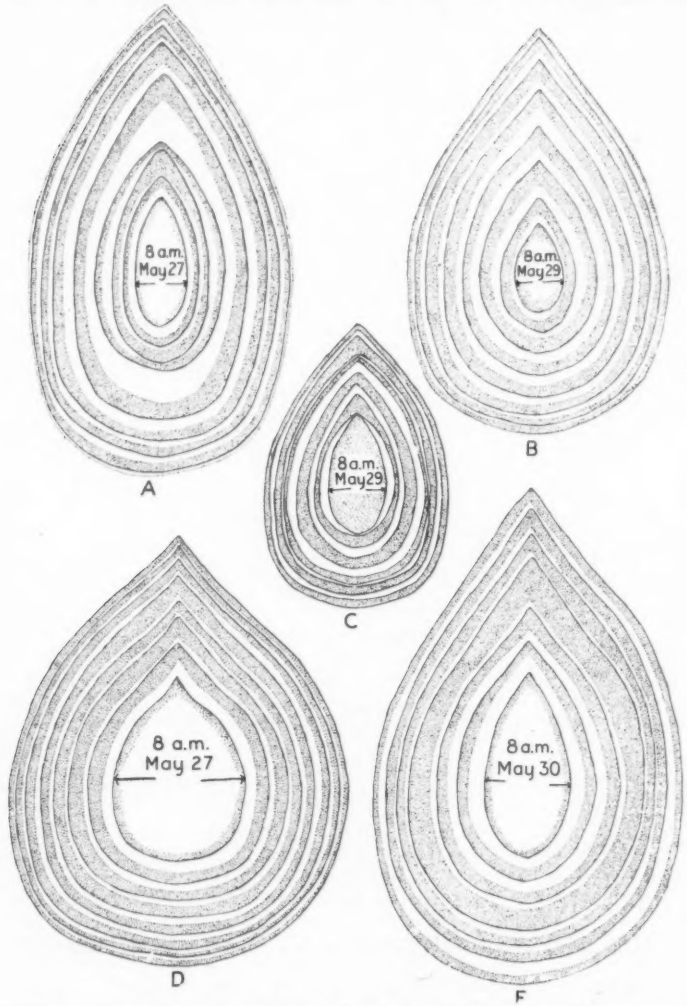

FIGURE 6.—Growth during day and night of five typical leaflets on plants in the greenhouse; daylight growth in white, night growth shaded

between day and night, although the night growth was a little greater. In certain leaflets the growth was quite regular; in others it was rather irregular. Occasionally it was greater at night, but frequently it was greater during the day. (Fig. 7.) No adequate explanation of this difference in the growth of individual leaves can be offered.

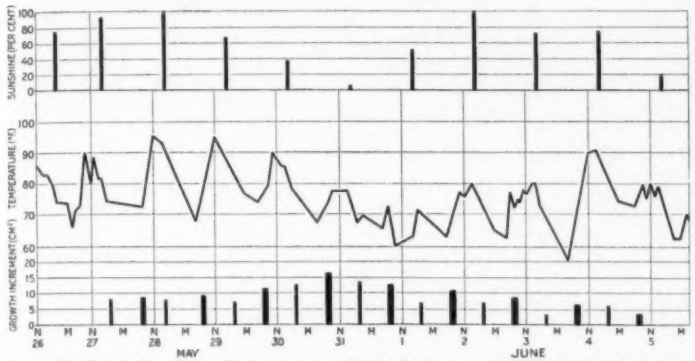

FIGURE 7.—Correlation of growth increments of 10 leaflets on greenhouse plants with temperatures and percentage of sunshine distributed about noon (N) and midnight (M). Night growths are shown by heavy and day growths by light black bars

TABLE 1.—*Area increase in square centimeters of three leaves on greenhouse plants by day and by night*

Leaflet and date	Leaf 1		Leaf 2		Leaf 3	
	Day	Night	Day	Night	Day	Night
Leaflet A:						
May 27	0.64	0.73	0.65	0.84	1.37	0.95
May 28	.67	.96	1.00	1.43	1.03	.84
May 29	.88	.37	1.34	1.83	1.09	1.20
May 30	1.59	1.27	1.93	3.05	1.91	2.00
May 31	.85	.90	.87	2.13	1.77	1.63
June 1	.64	.87	2.34	1.56	1.28	.87
June 2	.90	.73	.73	3.19	2.00	1.56
Total	6.17	5.83	8.86	14.03	10.45	9.05
Leaflet B:						
May 27	.26	.11	.33	.36	.17	.10
May 28	.15	.38	.35	.35	.37	.43
May 29	.28	.23	1.01	1.14	.36	.26
May 30	1.04	.57	.84	1.24	1.11	.87
May 31	.87	.89	1.45	1.49	.60	.67
June 1	.34	.59			.61	1.41
June 2	.34	.96			.05	.43
Total	3.28	3.73	3.98	4.58	3.27	4.17
Leaflet C:						
May 27	.14	.18	.15	.21	.15	.40
May 28	.26	.35	.17	.47	.37	.43
May 29	.52	.16	.49	.44	.52	.37
May 30	.20	.19	.47	.96	.72	.60
May 31	.39	.41	.35	.39	.42	.86
June 1			.39	.56	.23	.72
June 2					.37	.25
Total	1.51	1.29	2.02	3.03	2.78	3.63

The percentage of night growth of three leaves from greenhouse plants follows:

Leaf 1:	Per cent	Leaf 2:	Per cent	Leaf 3:	Per cent
Leaflet A	48.6	Leaflet A	61.3	Leaflet A	46.4
Leaflet B	53.2	Leaflet B	53.5	Leaflet B	56.0
Leaflet C	46.1	Leaflet C	60.0	Leaflet C	56.6
Average	49.3	Average	58.3	Average	53.0

Average night growth, nine leaflets, 53.5 per cent.
Average day growth of same leaflets, 46.5 per cent.

The average growth of 10 leaflets from three different plants during the same 8-day period was as follows: Day growth, 135 cm², or 45.9 per cent;[3] night growth 159 cm², or 54.1 per cent.

Night growth was slightly greater than day growth in spite of the fact that night temperatures were much lower than day temperatures. In the greenhouse the temperature was as low as 60° F. at night, whereas during the day it sometimes rose above 100° in full sunlight.

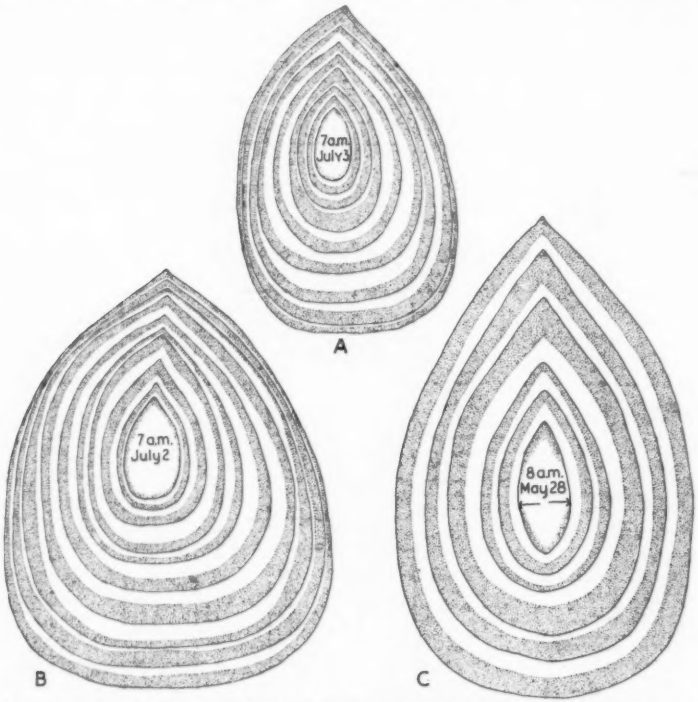

FIGURE 8.—Growth during day and night of three typical leaflets on plants grown out of doors; daylight growth in white, night growth shaded

The thermograph was shaded and so did not record the maximum temperature in full sunlight.

The relation of day and night growth to sunshine and temperature is shown in Figure 7. The sunshine records were obtained from the near-by weather bureau station at Burlington, Vt.; the temperature records were taken with a Friez recording thermograph. The period of maximum growth was from May 30 to June 2, the peak occurring on May 31. This period coincides with that of minimum sunshine and low temperatures both day and night. The days preceding this period had been almost cloudless and the temperature high both day and night, but the effect of these conditions was not shown by the

[3] Cm² is the abbreviation for square centimeter recently adopted by the Style Manual for United States Government printing.

leaves until a few days later. Apparently these leaflets had reached the period of maximum development, and the process continued in spite of slightly unfavorable surroundings, or perhaps the very favorable conditions for carbohydrate formation did not show in the plant until several days after they had passed. Fischer (*5*) in a study of the growth of rye plants found that favorable or unfavorable effects of temperature and moisture were not immediately apparent but were expressed by the plant in the period that followed and the stimulus of a favorable or unfavorable period varied directly as its length.

DAY AND NIGHT GROWTH OF LEAVES ON OUTDOOR PLANTS

Three leaves, each with a terminal leaflet and two pairs of side leaflets, were measured at 7 a. m. and at 7 p. m. At the beginning of the measurements the leaves were just entering the period of rapid growth. The records were kept as long as the measurements showed differences in day and night growth. The time selected, July 21 to 27, is the period of most rapid foliage growth in the field in this locality. The results of the measurements are given in Table 2. Figure 8 shows day and night growth in three typical leaflets chosen from supplementary series of measurements. In these leaflets also night growth was slightly in excess of day growth.

TABLE 2.—*Area increase in square centimeters of three leaves on outdoor plants, by day and by night*

Leaflet and date	Leaf 1		Leaf 2		Leaf 3	
	Day	Night	Day	Night	Day	Night
Leaflet A:						
July 21	1.64	3.63	0.52	4.74	1.73	3.62
July 22	1.94	3.82	3.11	3.00	4.00	3.18
July 23	0	4.51	.72	3.54	4.10	5.77
July 24	2.48	4.97	1.34	2.68	1.32	2.46
July 25	2.94	4.80	5.73	2.51	6.21	2.39
July 26	4.52	1.80	5.24	4.41	4.78	3.79
July 27	4.40	0	0	3.31	0	2.95
Total	17.92	23.53	16.66	24.19	22.14	24.16
Leaflet B:						
July 21	.52	.73	.43	1.56	.92	.62
July 22	1.08	1.04	.46	1.27	1.17	.84
July 23	1.27	.78	.71	.72	2.39	2.49
July 24	1.87	2.50	1.20	1.96	.75	1.48
July 25	1.54	4.10	1.81	1.57	2.76	2.91
July 26	2.66	3.25	1.97	2.12	4.97	2.52
July 27	.48	2.23	2.09	.62	0	3.38
Total	9.42	14.63	8.67	9.82	12.96	14.24
Leaflet C:						
July 21						
July 22	.49	0	.71	.23	.63	1.08
July 23	1.34	.58	.53	.96	.94	.66
July 24	.76	1.17	.50	2.04	1.37	1.45
July 25	.98	1.47	1.89	2.55	.76	2.42
July 26	1.08	1.51	2.51	1.79	2.32	1.81
July 27	1.57	.43	1.70	.72	.50	.88
Total	6.22	5.16	7.84	8.29	6.52	8.30

The percentage of night growth of three leaves from plants grown out of doors is as follows:

Leaf 1:	Per cent	Leaf 2:	Per cent	Leaf 3:	Per cent
Leaflet A	56.7	Leaflet A	59.2	Leaflet A	52.2
Leaflet B	60.8	Leaflet B	53.1	Leaflet B	52.3
Leaflet C	45.3	Leaflet C	51.4	Leaflet C	56.0
Average	54.3	Average	54.6	Average	53.5

Average night growth, nine leaflets, 54.1 per cent.
Average day growth of same leaflets, 45.9 per cent.

The average growth of 10 leaflets from three outdoor plants during the same 8-day period was as follows: Day growth, 167 cm^2, or 43.2 per cent; night growth, 220 cm^2, or 56.8 per cent.

The relative independence of growth and temperature is further emphasized by Figure 9. For example, the night temperatures of July 22–23, July 24–25, and particularly of July 25–26 were very low, but on these dates night growth was very rapid, and on the last-

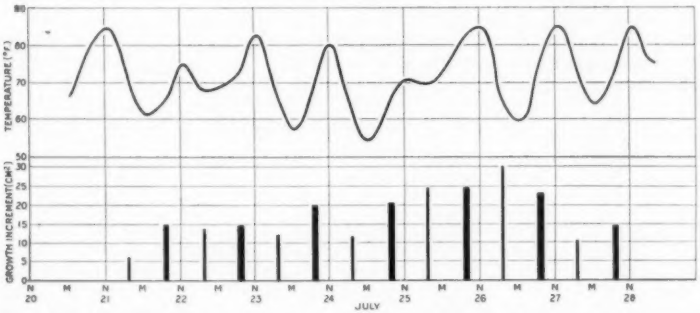

FIGURE 9.—Correlation of growth increment of 10 leaflets on plants out of doors, with daily temperatures distributed about noon (N) and midnight (M). Night growths shown by wide and day growths by narrow black bars

named date it was greater than for any other night period. Apparently growth, once started, proceeds almost in complete disregard, for a time at least, of the temperature of the environment.

As in leaflets on greenhouse plants, great irregularity occurred in the day and night growth of individual leaves and leaflets. Apparently internal factors are more important than external ones, at least in their immediate effects.

DISCUSSION

Decrease in the growth rate of each succeeding leaf is conditioned on the formation of a new leaf which removes part of the food supply. As soon as a new leaf is formed, the growth of the one below it is checked and the new leaf takes on accelerated growth. During the period of vegetative growth the wave of acceleration passes up the stem from one leaf to the next above it. In the potato this growth movement is not checked by the development of flowers, as very little material passes into them and they rarely set seed.

In the embryonal condition the leaf grows slowly, as the material for tissue formation must be drawn largely from older leaves through the stem, which serves as a common reservoir for food materials. As

soon as the young leaves are established, however, they constantly use more food, partly of their own manufacture and partly that made by the older leaves. Thus, the new leaves slow down the growth of the older ones. Some general factor must also be involved, probably that of permeability; the older membranes being less easily penetrated by solutions than the younger ones, the current of food takes the path of least resistance, i. e., into the younger organs.

SUMMARY

All leaves on a single potato shoot were measured at intervals for a period of about 20 days in the greenhouse during May and in the field in July.

Growth of leaflets and leaves followed the typical S-shaped curve. The leaves first formed stopped growth during the early part of the observations, and the next younger leaves then began rapid growth. At the close of the observations the youngest leaves were just beginning this period of accelerated growth.

The rate of growth of leaflets composing a single leaf is not always the same on any single day; so growth seems to be more or less independent of environment.

The growth of a shoot continues after the flower clusters are formed, a new vegetative shoot pushing them to one side. In this manner, the shoot continues to grow and the foliage to increase in spread.

Measurements on rapidly growing leaflets taken in the morning and at night showed: In greenhouse plants, night growth, 54.1 per cent; day growth, 45.9 per cent; in outdoor plants, night growth, 56.8 per cent; day growth, 43.2 per cent.

The accelerated growth of each leaf seems to be checked by the development of a newer leaf. The checking of vegetative growth in the potato plant, however, is not due to flower formation, for growth continues after flower clusters have appeared.

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